Russian Chemical Reviews **75** (12) 1049 – 1064 (2006) © 2006 Russian Academy of Sciences and Turpion Ltd

DOI 10.1070/RC2006v075n12ABEH003640

Certain aspects of the reactivity of carotenoids. Redox processes and complexation

N E Polyakov, T V Leshina

Contents

I.	Introduction	1049
II.	Redox reactions of carotenoids with quinones and metal ions	1051
III.	Reactions of carotenoids with free radicals. Antioxidant properties of carotenoids	1052
IV.	Pro-oxidant properties of carotenoids	1054
V.	The role of electron-transfer processes in the *cis* – *trans* isomerisation of carotenoids	1054
VI.	Inclusion complexes of carotenoids with cyclodextrins	1057
VII.	Supramolecular complexes of carotenoids with glycyrrhizin	1057
VIII.	Conclusion	1062

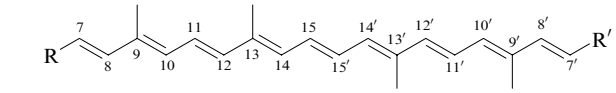
Abstract. The published data on the redox reactions of carotenoids, their supramolecular inclusion complexes and the composition, properties and practical application of these complexes are generalised. Special attention is given to the effect of complexation on radical processes involving carotenoids and on the antioxidant activity of carotenoids. The bibliography includes 151 references.

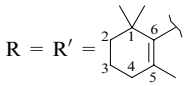
I. Introduction

Carotenoids, the natural pigments, are widely distributed in plants and animals.[1–3] They are detected in cells and tissues of all living things. Higher plants, fungi, bacteria and microbes can synthesise carotenoids, while animals and humans get them with food. In nature, carotenoids can exist in different states, namely, in the free state, as esters with fatty acids, glycosides and as carotene – protein complexes. To date, more than 600 different carotenoids are known.

Carotenoids belong to a large class of terpenes. A carotenoid molecule contains a long polyene chain of isoprene units with a cyclohexene (ionone) ring or isoprene residues at one or both ends. The majority of natural carotenoid pigments are represented by tetraterpenes (C_{40}). In plants, two groups of C_{40} carotenoids are usually present, namely, carotenes ('pure' hydrocarbons including β-carotene and its isomers, lycopene, see below) and xanthophylls (oxygen-containing carotenoids among which lutein, zeaxanthin, canthaxanthin, violaxanthin are the most abundant). Carotenoids with the number of carbon atom less than 40 (nor- and apocarotenoids, see below) and more than 40 (homocarotenoids) are also known.

Carotenoids play an important role in various physiological processes of critical importance for the life in its present form. In most natural processes, carotenoids are assumed to play the role of energy and electron carriers.[4] For plants, the involvement of carotenoids in the photosynthesis is of fundamental importance. In higher plants, the photosynthetic processes occur in chloroplasts, *i.e.*, specific energy-converting organelles of plant cells. Chloroplasts contain two photosystems, namely, PS1 and PS2, which differ in the composition of proteins and pigments and in

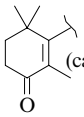


R = R′ =  (β-carotene);

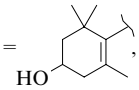
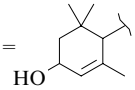
R = , R′ =  (α-carotene);

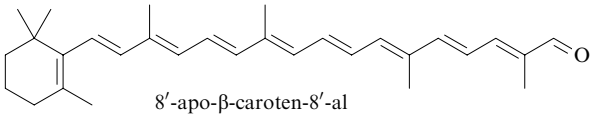
R = , R′ =  (γ-carotene);

R = R′ =  (ε-carotene); R = R′ =  (lycopene);

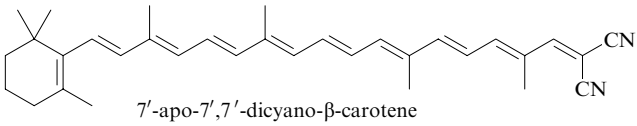
R = R′ =  (violaxanthin); R = R′ =  (canthaxanthin);

R = R′ =  (zeaxanthin);

R = , R′ =  (lutein).



8′-apo-β-caroten-8′-al



7′-apo-7′,7′-dicyano-β-carotene

N E Polyakov, T V Leshina Institute of Chemical Kinetics and Combustion, Siberian Branch of the Russian Academy of Sciences, ul. Institutskaya 3, 630090 Novosibirsk, Russian Federation. Fax (7-383) 330 73 50, tel. (7-383) 333 29 47, e-mail: polyakov@ns.kinetics.nsc.ru (N E Polyakov), tel. (7-383) 333 14 05, e-mail: leshina@ns.kinetics.nsc.ru (T V Leshina)

Received 10 April 2006
Uspekhi Khimii **75** (12) 1175 – 1192 (2006); translated by T Ya Safonova

photophysical properties. Photosystem 2 is responsible for the photosynthetic oxidation of water, while PS1 catalyses the oxidation of plastocyanine and the reduction of ferredoxin. Both systems include a light-collecting antenna (a pigment–protein complex that contains chlorophyll, carotenoid and protein molecules), a photochemical reaction centre, which consists of chlorophyll dimers (P700 in PS1 and P680 in PS2), electron donors and acceptors and electron-transfer molecules bound to this centre.

In the first step of photosynthesis, the absorption of light quanta by chlorophyll and carotenoid molecules converts them into the excited state, which is followed by energy transfer to other molecules of the photosystem. The second step involves the charge separation in the photochemical reaction centre of the PS2 and electron transfer along the electron-transport system from PS2 to PS1. Finally, ferredoxin, which is the electron carrier from PS1 to nicotinamide adenine dinucleotide phosphate (NADP), is reduced in PS1. The process ends with the reduction of NADP and synthesis of adenosine triphosphate (ATP).

According to modern concepts, the photochemical reaction that occurs in the PS2 reaction centres includes electron transfer from the excited molecule of chlorophyll P680 (its absorption spectrum is characterised by a peak at 680 nm) to a pheophytin molecule (PH, a primary electron acceptor) to form a state with separated charges ($P680^+\cdots PH^-$) followed by electron transfer from PH^- to a molecule of a secondary electron acceptor, namely, natural quinone (Q, a complex of plastoquinone with a Fe^{2+} ion) to afford a more stable state $P680^+\cdots PH\cdots Q^-$. The electron is transferred further by quinone carriers. Oxidised $P680^+$ exhibits exceptionally high electron affinity, *i.e.*, it is a very strong biological oxidant. If, for some reasons, $P680^+$ is not consumed in water oxidation, it can oxidise molecules in its nearest environment thus damaging PS2.

It should be noted that the risk of damaging the photosynthetic system as a result of side photochemical processes that occur in chloroplasts under the action of light (photoinhibition) is very high. Photoinhibition can be caused by different factors, including the formation of singlet oxygen. In chloroplasts, singlet oxygen is formed in the reaction of oxygen with excited chlorophyll molecules. Carotenoids present in chloroplasts uptake the excess energy from the excited chlorophyll molecules, which reduces the hazard of the formation of singlet oxygen. Thus, carotenoids are assumed to play the key role in the protection of organic molecules against oxidation.

In animal and human organisms, carotenoids perform different protective functions, *viz.*,[5–11]

— bind singlet oxygen and inhibit the formation of free radicals thus preventing their adverse effect on the organism;

— provide protection against UV radiation;

— play the role of antioxidants.

Of no less significance are the membrane-stabilising and immunostimulating functions of carotenoids as well as their provitamin A activity. Vitamin A and carotenoids favour normal metabolism, enhance the resistance of an organism against infections, provide normal operation of the organ of vision, exert a beneficial effect on the performance of skin and mucous membranes and are involved in redox processes.

Nowadays, a peak of interest in the studies of carotenoids is observed, being primarily associated with the discovery of their antioxidant properties. Now, it can be stated with confidence that it is the chemical and physicochemical studies of the reactivity of carotenoids in redox processes that are most promising. Scientists in different fields, namely, physics, chemistry, biology and medicine, are involved in the elucidation of the nature of the major factors that determine the antioxidant activity of carotenoids.[12–17] Despite the consensus on the elementary mechanisms of the antioxidant effect of carotenoids, no quantitative data on the relationship between the physicochemical properties of these compounds and their antioxidant activity are available in the literature.

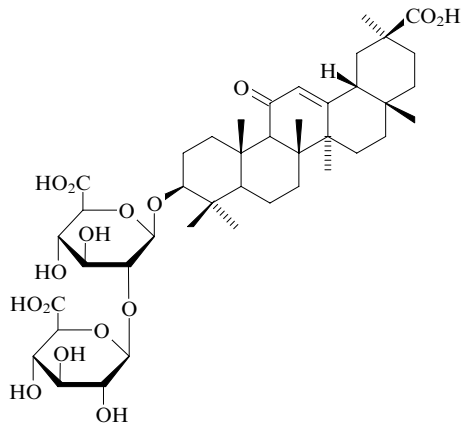
The information on short-lived paramagnetic and diamagnetic intermediates formed in reactions of carotenoids with free radicals is also very scarce,[14–17] and the structure of such intermediates is as a rule merely suggestive. However, it is this information that is the most valuable when selecting carotenoids for their practical application as antioxidants.† In recent years, due to the use of EPR techniques, considerable advance has been made in the studies of the redox processes involving carotenoids.[14, 18, 19]

Yet another important problem closely associated with the redox properties of carotenoids is the formation of carotenoid complexes and the effect of an organised medium on their reactivity in redox processes.

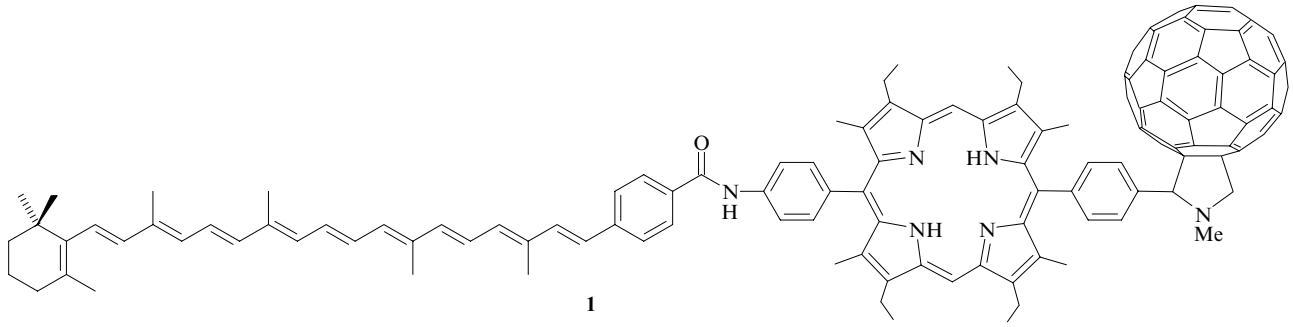
It is known that carotenoids are characterised by high lability in the free state and, at the same time, become more stable when being components of various complexes (*e.g.*, with proteins). Hydrophobicity, photosensitivity and chemical activity of free carotenoids substantially complicate their use as medicinal drugs; this is why active studies on the development of the methods for the synthesis of their complexes are in progress. Recently, a number of fundamental and applied studies on the synthesis and investigation of supramolecular inclusion complexes of carotenoids with different natural compounds, first of all, with cyclodextrins have appeared.[20–29] Cyclodextrins not only protect carotenoids from external factors, but also improve their solubility in water and organic solvents. Upon formation of inclusion complexes of the 'host–guest' type of cyclodextrins with size-matching hydrophobic inclusion compounds (*e.g.*, carotenoids), the physical, chemical and biological properties of the latter can substantially change (for details, see Refs 30–32).

In the pharmaceutical, cosmetic and food industries, cyclodextrins are largely used for improvement of solubility of inclusion complexes (*e.g.*, vitamins, food pigments, *etc.*[33–35]). Complexation can also substantially increase the stability and bioaccessibility of inclusion complexes, eliminate objectionable taste and odour, prevent side effects of medicinal drugs and favour their assimilation.[33, 35] Due to the ability of cyclodextrins to molecular recognition and separation of chiral isomers, they are used in the production of synthetic enzymes.[36, 37] However, complexation can also have some negative consequences. Thus the increase in the stability and solubility of carotenoids upon formation of inclusion complexes with cyclodextrins is accompanied by the loss of their antioxidant activity.

β-Glycyrrhizic acid (GA),[38] which belongs to triterpene glycosides, is yet another natural compound for which supramolecular inclusion complexes with carotenoids are described.



† The ability of carotenoids to decelerate ageing processes and to prevent and stop the development of diseases caused by reactive oxygen species and free radicals (superoxide ion, singlet oxygen, *etc.*) are actively discussed in the literature. Carotenoids are used for treatment of serious illnesses such as cancer and cardiac and nervous system diseases and age-specific degenerative changes in muscular tissues.



1

Complexes of biologically active compounds with GA attract attention of scientists due to their unusually high stability [39–41] and due to the fact that the activity of these compounds in such complexes increases by a factor of dozens‡ with simultaneous reduction of their toxicity.[39, 42]

It should be noted that despite the interest in the synthesis of inclusion complexes aimed at purposeful changes in their properties, the problem of the nature of the effect of complexation on their reactivity is still open. The present survey of the studies that deal with single-electron transfer processes involving carotenoid complexes is one of few attempts to solve this problem.

The purpose of this review is to briefly generalise the studies of the redox properties of carotenoids, their complexation and reactions of these complexes. Carotenoid reactions that include the electron transfer strep are described in more detail. The relationship between the efficiency of single-electron transfer and the antioxidant/pro-oxidant activity of carotenoids and their supramolecular complexes is discussed.

II. Redox reactions of carotenoids with quinones and metal ions

A long chain of conjugated double bonds in a carotenoid molecule provides the electron-donating activity of carotenoids and favours stabilisation of their radical forms.[43] Studying of physicochemical properties of carotenoid radical cations attracts the keenest attention of scientists, attempting to model the system of primary photosynthetic processes§ involving carotenoids, aimed at the development of artificial solar cells.[44–46] Successful solution of this problem is closely related to the efficient spatial separation of charges in an artificial photosystem involving carotenoids and porphyrins. In connection with this, the elucidation of how an organised medium affects the structure of carotenoid itself and the lifetimes and chemical properties of radical cations acquires special importance.

The studies of an isolated PS2 have shown[47] that its photoactivation includes selective oxidation of β-carotene accompanied by electron transfer from the carotene molecule (Car) to the primary donor $P680^{+\bullet}$ to form a radical cation $Car^{+\bullet}$. The formation of the radical cation $Car^{+\bullet}$ in the irradiation of PS2 was detected by optical absorption spectroscopy[48–50] and low-temperature EPR,[51, 52] while the formation of quinone radical anions in PS1 and PS2 was detected by the EPR method.[53–55] As it has been mentioned above, in PS2, the electron-transfer chain involves molecules of carotenoids, chlorophyll, pheophytin and natural quinones [plastoquinones, ubiquinones (Q)]. This is why triads of the $[Car^{+\bullet}\cdots P\cdots Q^{-\bullet}]$ type are also used in model electron-transfer systems.[56–58] The use of a molecular triad carotenoid – porphyrin – fullerene (**1**) was a windfall.[58]

The long lifetime of triads opens up wide possibilities for their use in molecular electronics as nanosized communication and data processing constructs in sensors. The principle of operation of molecular triads is rather simple. A porphyrin molecule excited upon absorption of a light quantum donates an electron to the fullerene molecule to form a primary radical-ion pair $[Car\cdots P^{+\bullet}\cdots C_{60}^{-\bullet}]$. Being a strong oxidant (electron acceptor), the radical cation $P^{+\bullet}$ abstracts an electron from a carotenoid molecule to form a separated radical-ion pair $[Car^{+\bullet}\cdots P\cdots C_{60}^{-\bullet}]$. This state is relatively long-lived and retains a considerable part of light energy by increasing the electrochemical potential.[58] Apparently, such a way of light energy transformation is similar to the mechanism used by plants for utilisation of solar energy in photosynthesis.

The formation of carotenoid radical cations in reactions with quinones in homogeneous media was observed both in the light and in the dark [using such a strong electron acceptor as 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ)].[59] In the first step, a charge-transfer complex (CTC) formed, which was followed by the formation of a radical-ion pair $[Car^{+\bullet}+Q^{-\bullet}]$. Carotenoid-to-quinone adducts (Car–Q) and carotenoid *cis*-isomers¶ were the final products.

$$Car + Q \longrightarrow CTC \longrightarrow [Car^{+\bullet} + Q^{-\bullet}] \longrightarrow Car{-}Q + \textit{cis}\text{-}Car$$

The formation of CTC in reactions of β-carotene, cantha-xanthin and 8′-apo-β-caroten-8′-al with quinones was detected by optical spectroscopy (Fig. 1) and EPR.[59] Thus the intense absorption at 1030 nm observed upon mixing of β-carotene with quinone in CH_2Cl_2 was assigned to a charge-transfer complex, while the free radical cation of β-carotene absorbs in this solvent at

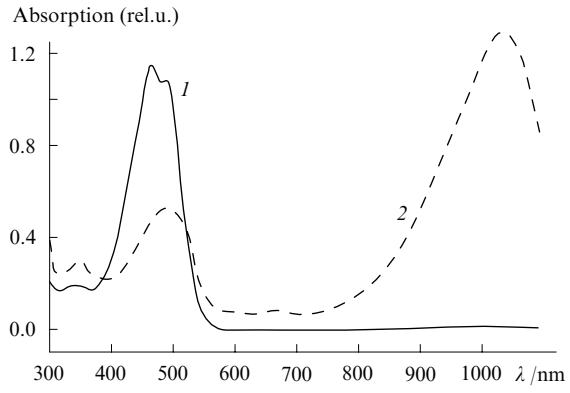


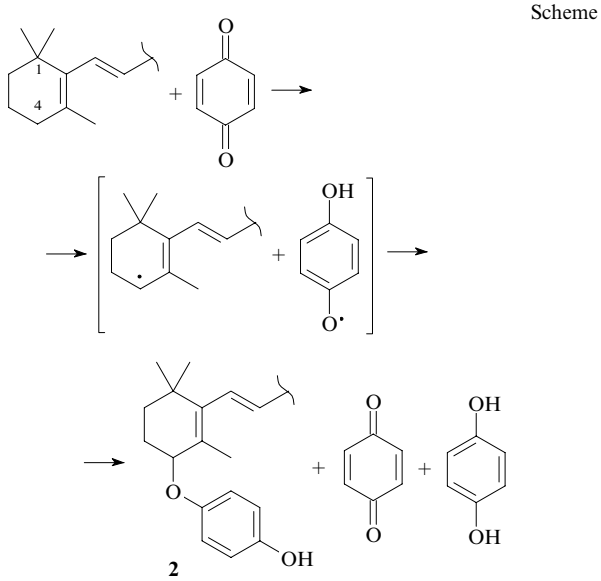
Figure 1. Absorption spectra of β-carotene and its CTC with quinone recorded before (curve *1*) and after (curve *2*) their mixing with DDQ in CH_2Cl_2 at −50 °C.[59] The concentrations of β-carotene and DDQ are 12 μmol litre^{-1}.

‡ The stability constant of GA complexes is 10^5 litre mol^{-1}, which exceeds the mean stability constant of cyclodextrin complexes by two orders of magnitude.[30]

§ At present, photosynthesis is one of the most thoroughly experimentally studied biological processes.

¶ Dissociation of radical – ion pairs escaped from recombination to free radical ions and their subsequent diffusion into the bulk are accompanied by *cis*–*trans* isomerisation of carotenoid radical cations to form *cis* isomers (see Section V).

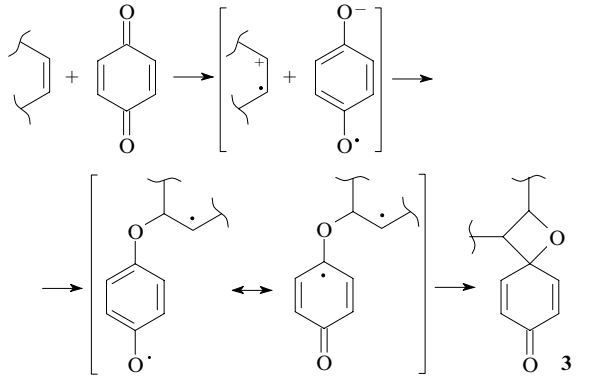
Scheme 1



1000 nm.[60] Further transformation of CTC might follow two pathways depending on the carotenoid structure. For β-carotene and 8′-apo-β-caroten-8′-al, the preferential reaction pathway is the abstraction of the allylic proton at C(4) atom of the cyclohexene ring to form a neutral carotenoid radical (Scheme 1).[59]

Ether **2** is the final product of this reaction. Its formation was confirmed by IR and NMR spectroscopy.[59] In canthaxanthin, no abstraction of the allylic proton is possible and the reaction proceeds through the addition of quinone to the chain of conjugated double bonds of the carotenoid (Scheme 2).

Scheme 2



[The photoinduced cycloaddition of a carbonyl group (including that of quinone) to the double bond of the polyene molecule is known as the Paterno – Büchi reaction.[61,62]] It is assumed that the first pathway of this process also involves electron transfer from a Car molecule to Q to form a biradical, adduct **3** being the final reaction product.[63,64]

Metal ions can also be used for generation of radical cations of carotenoids in both homogeneous and organised media.[65–67] The mechanism of the redox reaction of carotenoids with metal ions in a homogeneous medium was studied in detail by the example of the reaction of β-carotene with iron(III) chloride.[43,65] The first stage was shown to be electron transfer from the Car molecule to Fe^{3+} to form a Fe^{2+} ion and a radical cation $Car^{+\bullet}$. Recombination of the radical cations affords a dication and a neutral molecule.

$$Car + Fe^{3+} \rightleftharpoons Car^{+\bullet} + Fe^{2+},$$

$$2\,Car^{+\bullet} \rightleftharpoons Car^{2+} + Car.$$

As in the above-discussed reaction with quinones, the carotenoid radical cations and dications formed undergo *cis–trans* isomerisation.[43,65,68,69] The oxidation of β-carotene with metal ions in the presence of oxygen affords a stable product, *viz*., 5,8-peroxide.[65]

The oxidation of carotenoids with metal ions in organised media was studied.[66,67,70] In particular, by the example of β-carotene, canthaxanthin and 8′-apo-β-caroten-8′-al, it was shown that in metal-containing mesopores of MCM-41 these carotenoids form compact complexes with Cu^{2+}, Ti^{4+} and Fe^{3+} ions in which the distance between the metal ion and the nearest double bond of the carotenoid is ~2 Å.[68,70] The formation of these complexes results in acceleration of both the direct and reverse electron transfer between the carotenoid and the metal and in the increase in the quantum yield of the formation of carotenoid radical cation.

III. Reactions of carotenoids with free radicals. Antioxidant properties of carotenoids

The interest in the reactions of carotenoids with free radicals is aroused first of all by the discovery of their antioxidant properties. A comprehensive description of carotenoid reactions with peroxide radicals can be found in recent reviews;[15–17] threfore, here we consider only the proposed mechanisms of these reactions. The role of electron transfer and its effect on the antioxidant activity of carotenoids will be considered in more detail.

It is known[71,72] that in a living organism, peroxide radicals are formed during chain autoxidation of lipids.

$$X^{\bullet} + RH \longrightarrow R^{\bullet} + XH \text{ (chain initiation)},$$

$$\left.\begin{aligned} R^{\bullet} + O_2 &\longrightarrow ROO^{\bullet}, \\ ROO^{\bullet} + RH &\longrightarrow ROOH + R^{\bullet} \end{aligned}\right\} \text{(chain propagation)},$$

$$ROO^{\bullet} + ROO^{\bullet} \longrightarrow [ROOOOR] \text{ (chain termination)}.$$

Here, X is a free radical that initiates the radical chain reaction, RH is a lipid molecule. We do not consider possible sources of free radicals in the organism and only refer to the study by Chan[72] in which the oxidation stress initiated by metal ions was assumed to be the main 'source' of free radicals. The process of free radical formation can be interrupted as a result of the chain termination by any antioxidant capable of reacting with peroxide radicals.[3,71–75] The reaction product is an antioxidant radical, which is less active than the starting peroxide radical. The low activity of the former makes impossible the chain propagation. Chain termination occurs as a result of the reaction of the antioxidant radical with either another radical or another antioxidant.

In the past decades, attempts were undertaken to compare the antioxidant activities of different carotenoids and to reveal the factors responsible for this activity.[14–17] This required the elucidation of the detailed mechanism of the reaction of carotenoids with free radicals, first of all, peroxide radicals.

The following three pathways for the reaction of carotenoids with peroxide radicals were considered most often: addition of a free radical to a double bond of a carotenoid [reaction (1)], abstraction of a hydrogen atom from the allylic fragment of a carotenoid (2) and electron transfer from a carotenoid to a peroxide radical to form a carotenoid radical cation (3).[7,76–78]

$$ROO^{\bullet} + Car \longrightarrow {}^{\bullet}Car{-}OOR, \quad (1)$$

$$ROO^{\bullet} + Car \longrightarrow Car^{\bullet} + ROOH, \quad (2)$$

$$ROO^{\bullet} + Car \longrightarrow ROO^{-} + Car^{+\bullet}. \quad (3)$$

(It should be mentioned that the radicals $ROOCar^{\bullet}$ and $Car^{\bullet}$ can in turn serve as the sources for the chain propagation of lipid oxidation with peroxides.) The analysis of the available literature data does not allow one to single out the major mechanism.[15–17,79]

It can be stated that the direction of the reaction of peroxide radicals with carotenoids depends on several factors, namely, the carotenoid structure, the activity of peroxide radicals and the nature of the solvent. Thus the analysis of absorption spectra and reaction products of β-carotene with peroxide radicals led to a conclusion that in non-polar media both a radical adduct $^{\bullet}$Car–OOH (Refs 80–83) and a radical $^{\bullet}$Car (Refs 84 and 85) are formed as the short-lived intermediates. However, the relative contributions of these reaction channels were not determined.

More detailed information on the mechanism and kinetics of the reaction of carotenoids with free radicals was obtained from the results of time-resolved pulse methods, first of all, flash laser photolysis and pulse radiolysis.[7, 8, 86, 87] However, these methods did not provide the complete picture of the reaction of carotenoids with free radicals either. To date, the absorption spectra are adequately characterised only for carotenoid radical cations,[88, 89] whereas the assignment of other signals in absorption spectra of intermediate species still remains hypothetical.

Based on studies of the reaction of β-carotene with the $Cl_3COO^{\bullet}$ radical in polar media, it was concluded[16] that this reaction affords equal amounts of radical cations and radical adducts of β-carotene. Similar conclusions were also drawn based on the analysis of absorption spectra of intermediate species formed in the laser-induced photolysis of certain carotenoids in micellar solutions.[7] In the latter case, the absorption spectra contained two bands in the region of 750–950 nm, which differed in the decay rate. The absorption in the range of 850–950 nm was assigned to carotenoid radical cations and the band in the region of 750–850 nm was assigned to the radical adduct.[7] However, a different assignment was proposed.[90, 91] In alcohols, the measurements of relative intensities of absorption bands in the regions of 750–850 and 850–950 nm have revealed a strong dependence of the signal intensity on the polarity of the medium in the former region.[91] Thus, it was concluded that the observed intermediate is most likely a radical-ion pair of carotenoid and peroxide anion.

It is known that carotenoids reside preferentially in the lipophilic region of the membrane due to their hydrophobicity.[76, 92] For a non-polar β-carotenoid incorporated into a membrane, the formation of a radical cation is improbable, because electron transfer in a non-polar medium is unfavourable for energy reasons. At the same time, carotenoids with polar substituents (*e.g.*, zeaxanthin and astaxanthin) are located at the membrane surface where the electron transfer is more favourable. Hence, the interaction of such carotenoids with radicals can easily lead to the formation of radical cations.[77, 93]

The data obtained by EPR spin-trapping techniques on the rate constants for the reaction of carotenoids with free radicals suggest that electron transfer is the primary step.[14] By the example of certain natural and specially synthesised carotenoids, it was shown that the scavenging rates of $^{\bullet}$OOH radicals by carotenoids correlate with the oxidation potentials of the latter (Fig. 2).[14] Stable spin adducts of $^{\bullet}$OOH radicals with spin traps formed in the absence and in the presence of carotenoids and their yields were measured. In the presence of a carotenoid, the yield of the spin adduct decreased as a result of the side reaction of hydroperoxide radical with the carotenoid (Fig. 3). Based on the experimental dependence of the yield of the spin adduct on the carotenoid concentration, the relative rate constants (k_{Car}/k_{ST}) for scavenging of radicals by the carotenoid were calculated according to Eqn (4).

$$\frac{A_0}{A} = \frac{k_{ST}[ST] + k_{Car}[Car]}{k_{ST}[ST]} . \qquad (4)$$

Here, A_0 and A are the intensities of the spin adduct signal in the absence and in the presence of a carotenoid, k_{ST} and k_{Car} are the rate constants for radical scavenging by a spin trap (ST) and by a carotenoid (Car), respectively. *N-tert*-Butyl-α-phenylnitrone (PBN), 2-methyl-2-nitrosopropane (MNP) and 5,5-dimethylpyrrolidine *N*-oxide (DMPO) were used as the spin traps.[14] The available kinetic database (Spin Trap Data Base; http://epr.niehs.nih.gov) contains the following value for the reaction of PBN with the hydroperoxide radical in water: $k_{ST} \leqslant 10^6$ litre mol^{-1} s^{-1}. The slowest and the highest reaction rates were observed for β-carotene and ethyl 8′-apo-β-caroten-8′-oate, respectively. Based on the comparison of the rate constants for the reactions of carotenoids with the hydroperoxide radical, it was concluded that reactions (1) and (2) make no substantial contribution to the reaction of carotenoids with these radicals.

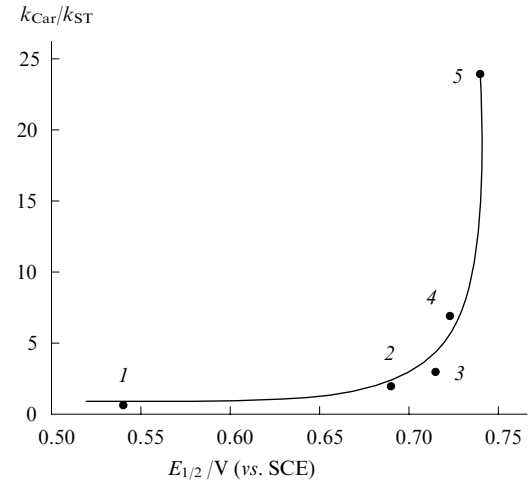


Figure 2. Dependence of the rate constant for scavenging of peroxide radicals by carotenoids on the oxidation potential of carotenoids (k_{Car} and k_{ST} are the rate constants for scavenging of the $^{\bullet}$OOH radical by carotenoids and by spin traps, respectively).[14]
Carotenoids: β-carotene (*1*), canthaxanthin (*2*), 8′-apo-β-caroten-8′-al (*3*), 7′-apo-7′,7′-dicyano-β-carotene (*4*), ethyl 8′-apo-β-caroten-8′-oate (*5*).

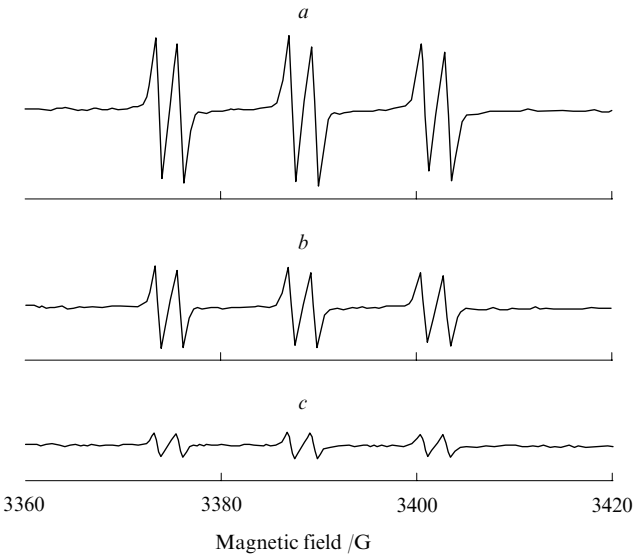


Figure 3. EPR spectra of the adduct PBN–OOH (PBN is *N-tert*-butyl-α-phenylnitrone) recorded in the absence (*a*) and in the presence of 1.2 mmol litre^{-1} (*b*) or 4.2 mmol litre^{-1} (*c*) of canthaxanthin in DMSO.[14] [PBN] = 5 mmol litre^{-1}, [$FeCl_2$] = 1 mmol litre^{-1}, [H_2O_2] = 0.5 mol litre^{-1}.

IV. Pro-oxidant properties of carotenoids

Clinical trials of β-carotene gave contradictory results, which could be a reason for ambiguous attitude to carotenoids as antioxidants.[94–96] Thus β-carotene demonstrated good results in curing certain types of tumours [97–100] and at the same time proved to be ineffective or even exhibited pro-oxidant † activity in curing certain other diseases caused by free radicals.[94–96]

It was shown that β-carotene exhibits the antioxidant activity only in low concentrations and at low oxygen pressures, whereas in high concentrations and at high oxygen pressures it exhibits the pro-oxidant activity.[6, 9, 11, 17, 101]

Neutral radicals Car$^{\bullet}$ and ROOCar$^{\bullet}$ as well as radical cations Car$^{+\bullet}$, which are formed in reactions (1)–(3), were assumed to be responsible for the pro-oxidant activity of carotenoids.[102–104] The mechanism of the pro-oxidant effect of carotenoids involves the reactions of neutral radicals of carotenoids Car$^{\bullet}$ and ROOCar$^{\bullet}$ with oxygen molecules to form the corresponding peroxide radicals

$$Car^{\bullet} + O_2 \longrightarrow CarOO^{\bullet},$$

$$ROOCar^{\bullet} + O_2 \longrightarrow ROOCarOO^{\bullet}.$$

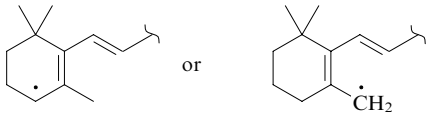
Neutral carotenoid radicals are also formed in the protonation/deprotonation of the corresponding radical ions. Thus the formation of neutral radicals of β-carotene, zeaxanthin, canthaxanthin, 8′-apo-β-caroten-8′-al and 7′-apo-7′,7′-dicyano-β-carotene and their structural analogue, β-ionone, was registered [18] by the EPR spin-trapping technique. The formation of neutral radicals Car$^{\bullet}$ was observed in chemical or electrochemical oxidation of these carotenoids to radical cations, which was followed by deprotonation of the latter [reaction (5)],[18] and in their electrochemical reduction or a reaction with piperidine. The latter reaction proceeds through the protonation of the corresponding radical anions [reaction (6)].[18]

$$Car^{+\bullet} \rightleftharpoons Car^{\bullet} + H^{+}, \quad (5)$$

$$Car^{-\bullet} + H^{+} \rightleftharpoons Car^{\bullet}. \quad (6)$$

The formation of carotenoid radicals was judged from hyperfine coupling (HFC) constants of the corresponding spin adducts obtained in simulations of the EPR spectra.

It was shown that the ability of carotenoid radical ions to undergo protonation/deprotonation is more typical of non-symmetrical apo-carotenoids with electron-withdrawing terminal groups. The calculation of the energy of deprotonation of carotenoid radical cations made it possible to assume that the abstraction of a hydrogen atom occurs from either the C(4) atom or the methyl group in position 5 of the cyclohexene ring. In both cases, the corresponding neutral radicals are formed.[18]

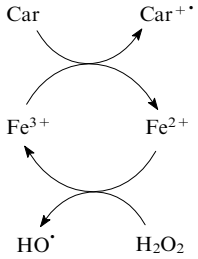


The reaction of carotenoids with metal ions, *e.g.*, with iron ions, may be considered as yet another possible mechanism of the pro-oxidant activity of carotenoids.[103, 105] As was noted above, the oxidation stress initiated by metal ions is one of the major sources of free radicals in the organism [72] and can result in the development of certain serious disorders including cancer.[106, 107] It is assumed that in this case, the mechanism of free radical generation is similar to the well-known Fenton reaction.[108]

$$Fe^{2+} + H_2O_2 \rightleftharpoons Fe^{3+} + {}^{\bullet}OH + HO^{-}, \quad (7)$$

$${}^{\bullet}OH + RH \longrightarrow H_2O + R^{\bullet}.$$

By the example of β-carotene and canthaxanthin, its was shown experimentally that the involvement of a carotenoid in this reaction results in an increase in the yield of free radicals due to the chain reaction.[105]



Reactions of carotenoids with peroxide and carbon-centred radicals were studied.[105] Based on the experimental results,[14, 105] it can be assumed that, in the presence of metal ions, carotenoids can either decrease or increase the yield of free radicals depending on the oxidation potential of the carotenoid and the nature of the radical. The efficiency of the carotenoid-induced Fenton reaction increases as the oxidation potential of the carotenoid decreases and its reaction with the radical decelerates. According to Polyakov and co-workers' data,[105] β-carotene, which has the lowest oxidation potential among all the carotenoids studied, exhibits the highest pro-oxidant activity in the Fenton reaction (note that it is for β-carotene that the highest pro-oxidant activity was observed *in vivo* [6, 94–96]).

V. The role of electron-transfer processes in the *cis–trans* isomerisation of carotenoids

Light-induced *cis–trans* isomerisation of carotenoids is one of characteristic pathways of their phototransformations.[109–113] It is usually meant that the isomerisation process involves molecules in the excited triplet state. [112, 113] For the processes of sensitised *cis–trans* photoisomerisation, the formation of the excited triplet state is possible as a result of transfer of either energy or an electron.[113–115] The latter process occurs in polar media in the presence of electron donors or electron acceptors to afford the corresponding radical ions. In connection with this, studies of the properties of radical ions of natural polyenes acquire special importance, because these compounds are incorporated in many biological systems.[4, 45, 116] Thus *cis*-retin-11-al is the chromophore of the visual pigment rhodopsin. It is assumed that its *cis–trans* isomerisation initiates the visual perception process in eyes of humans and mammals.[117, 118] This is the first and the only photochemical reaction that triggers the primary vision process, namely, phototransduction. Certain *cis*-carotenoids play an important role in photosynthetic processes in bacteria.[119]

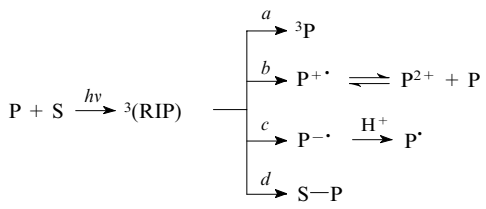
Let us consider in more detail the mechanisms of *cis–trans* isomerisation of polyenes that involve the electron-transfer step. The major part of results discussed here were obtained using the following methods of spin chemistry:[120] chemically induced dynamic nuclear polarisation (CIDNP) and magnetic field effect (MFE) methods. These methods are the most efficient for studying the mechanisms of complex reactions of radicals and radical ions.‡

It has now been shown experimentally that *cis–trans* isomerisation of polyenes (P) that proceeds in the presence of electron donors or electron acceptors (S) can involve the following short-

† The pro-oxidant activity of a compound usually implies the increase in the yield of active oxygen-centred radicals in the presence of this compound.

‡ It should be noted that it is the methods of spin chemistry that have provided evidence for the occurrence of single-electron transfer in *cis–trans* photoisomerisation of substituted ethylenes in the presence of electron donors or electron acceptors. This is acknowledged among the most important achievements of spin chemistry.[121, 122]

Scheme 3



P are polyenes (carotenoids, β-ionone), S are electron donors or electron acceptors (amines, quinones, aromatic hydrocarbons).

lived species (Scheme 3):[114] excited triplet states (^{3}P) formed in the recombination of a triplet radical–ion pair (RIP) (pathway *a*); radical cations ($P^{+\bullet}$) and dications (P^{2+}) (pathway *b*); neutral radicals ($P^\bullet$) and biradicals (P–S adducts), *i.e.*, secondary intermediates formed from radical ions (pathways *c* and *d*, respectively).

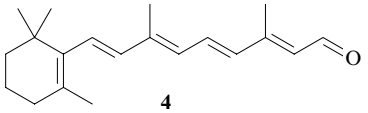
The formation of excited triplet states was observed in the isomerisation of substituted ethylenes and β-ionone in the presence of amines and aromatic hydrocarbons. Radical cations are formed in the presence of substituted ethylenes and carotenoids. Neutral radicals and biradicals are formed in the isomerisation of β-ionone in the presence of quinones.

1. *cis–trans* Isomerisation of alkenes in the excited triplet state

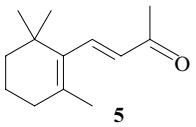
Triplet states of alkenes are formed either upon UV irradiation (direct isomerisation) or as a result of energy transfer (sensitised isomerisation).[123] There is the third mechanism of isomerisation of triplet alkenes established using the methods of spin chemistry.[121, 122] According to this mechanism, triplet molecules are formed as a result of reverse electron transfer in a triplet RIP formed in the irradiation of substituted ethylenes in the presence of electron donors or electron acceptors in polar media.

In contrast to substituted ethylenes, polyenes are represented by a larger number of isomers, which often differ in the energy of the triplet states, redox potentials and their reactivities with respect to electron donors and electron acceptors.

Retinal (**4**) and β-ionone (**5**) were the first polyenes of the terpene series for which the radical ion mechanism of photoisomerisation in the presence of electron donors or electron acceptors was confirmed. [115, 124, 125]

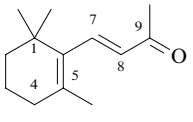


4

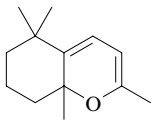


5

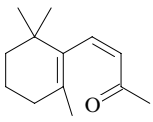
Let us consider how the isomerisation of β-ionone proceeds in the presence of electron donors, *viz.*, pyrene or triphenylamine.[115] Four β-ionone isomers, namely, (*E*)-β-ionone (**A**), α-pyran (**B**), (*Z*)-β-ionone (**C**) and (*Z*)-*retro*-γ-ionone (**D**) are known.[126]



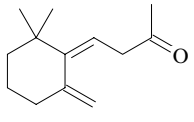
A



B



C



D

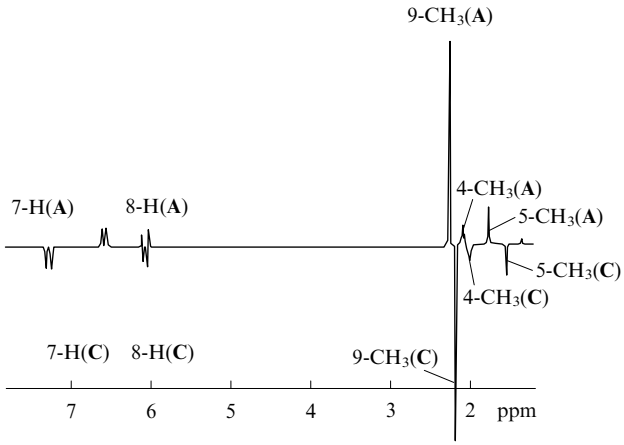
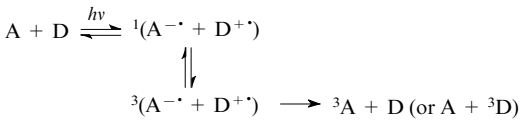


Figure 4. CIDNP spectrum observed in the photolysis of a photostationary mixture of β-ionone isomers in the presence of triphenylamine in acetonitrile.[115] A procedure of presaturation of the NMR spectrum was used for elimination of equilibrium signals.

NMR studies have shown that the kinetics of accumulation and the ratio of isomers formed in the irradiation of β-ionone in the presence of pyrene and triphenylamine substantially differ from those in direct photolysis.[115] During the photolysis of β-ionone in the NMR tube, the CIDNP effects of the main isomers were registered. Figure 4 shows the CIDNP spectrum recorded in the photolysis of a mixture of β-ionone isomers in the presence of triphenylamine. The mechanisms of transformations of β-ionone isomers could be proposed from the analysis of this spectrum.

In the CIDNP spectrum observed during the irradiation of a photostationary mixture of isomers **A**–**D** in the presence of triphenylamine, only signals for the triplet isomers **A** and **C** were detected. This made it possible to conclude that *cis* isomer **C** is the transformation product of the excited triplet *trans* isomer **A** and *vice versa*. Triplet states were formed in the recombination of the corresponding radical-ion pairs (Scheme 4).[115] In turn, it was shown that in the presence of triphenylamine, isomers **B** and **D,** namely, α-pyran and *retro*-γ-ionone, are not formed *via* RIP.[114, 115] However, in the presence of pyrene, the formation of a RIP with α-pyran as the radical cation and pyrene as the radical anion was observed.

Scheme 4



A is an electron acceptor, D is an electron donor.

The CIDNP sign and the intensities of polarised lines corresponded to the signs and distribution of HFC constants (*a*) in the β-ionone radical anion ($a_4 = 1.2$, $a_5 = 0.65$, $a_7 = -1.0$ and $a_9 = 0.65$ mT).[127] It should be noted that Scheme 4 is versatile, *i.e.*, it can be used for describing the reactions of β-ionone with both electron acceptors (*e.g.*, quinones) [125] and electron donors (*e.g.*, pyrene and triphenylamine).[115] β-Ionone plays the role of the electron donor in the former case and the electron acceptor in the latter case.

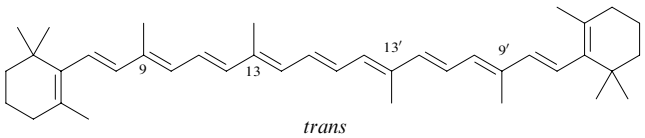
This example shows that CIDNP is indeed an effective tool for studying the geometrical isomerisation of polyenes that involves radical stages.

2. *cis–trans* Isomerisation of C=C bonds in radical ions of polyenes

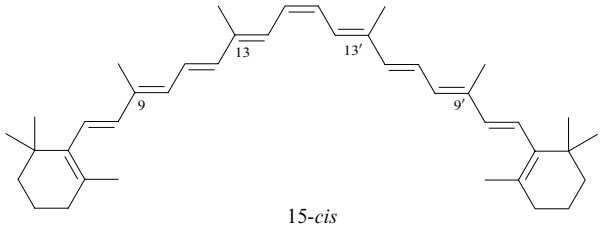
cis–trans Isomerisation of radical ions was described for the first time also for substituted ethylenes.[122, 128, 129] The CIDNP method did not allow us to observe the isomerisation of radical ions of short polyenes, namely, of β-ionone (**5**) and retinal (**4**), in the microsecond time interval suitable for the CIDNP manifestation.[124, 125] This means that even if the radical ions of these polyenes isomerise, this process takes much longer time than the microsecond interval of the lifetime of short-lived RIP. Indeed, as follows from quantum-chemical calculations, the activation barrier to the *cis–trans* isomerisation of β-ionone radical ions is 10–20 kcal mol^{-1}, which corresponds to the isomerisation rate of $\sim 10^4$ s^{-1}. However, we managed to observe the *cis–trans* isomerisation of radical cations of β-carotene, canthaxanthin and 8′-apo-β-carotenal generated either chemically or electrochemically.[65, 68, 130, 131] These observations became possible due to the longer lifetimes of these radical cations as compared with those of β-ionone and retinal. Thus the lifetimes of radical cations of β-carotene and canthaxanthin in dichloromethane solutions were equal to several minutes.[59, 132] HPLC analysis of the electrolysis products of β-carotene indicated the formation of five isomers, namely, *trans*, 15-*cis*, 13-*cis*, 9-*cis* and 9,13-di-*cis*, in a ratio of 50 : 4 : 21 : 21 : 4.[59, 132] This isomer ratio was observed for both the electrochemical generation of radical cations and chemical oxidation of carotenoids in the presence of $FeCl_3$ or quinones.

It should be emphasised that this ratio substantially differs from that obtained by Hashimoto and Kayama [133] for the triplet-sensitised isomerisation of β-carotene. Under photostationary conditions, these authors observed only four isomers, namely, *trans*, 15-*cis*, 13-*cis* and 9-*cis*, in a ratio of 84 : 1.5 : 4 : 10.5.

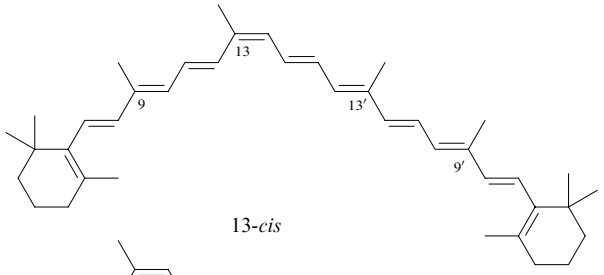
According to AM1 calculations,[134] the energy barriers to isomerisation of bonds in the β-carotene radical cation ($\sim$20 kcal mol^{-1}) and the dication ($\sim$0 kcal mol^{-1}) were substantially lower than those of the neutral molecule ($\sim$55 kcal mol^{-1}). The calculated [134] lengths of C=C bonds in the middle part of the conjugated chain substantially differ for the neutral molecule, the radical cation and the dication: they change from 1.35 Å in the neutral molecule to 1.40 Å in the radical cation and to 1.45 Å in the dication. The latter value corresponds to the length of a single C–C bond in a carotenoid molecule.



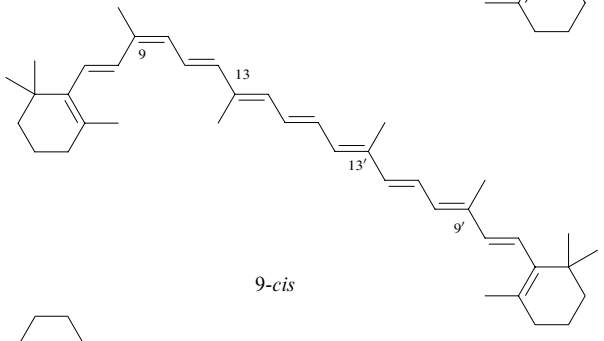
trans



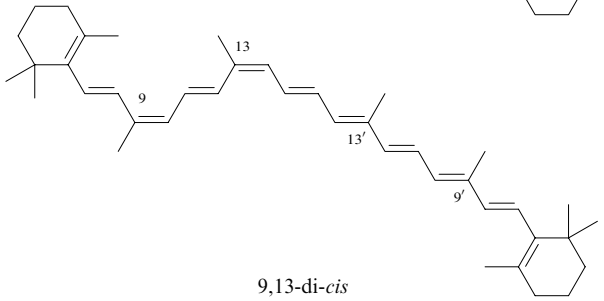
15-*cis*



13-*cis*



9-*cis*

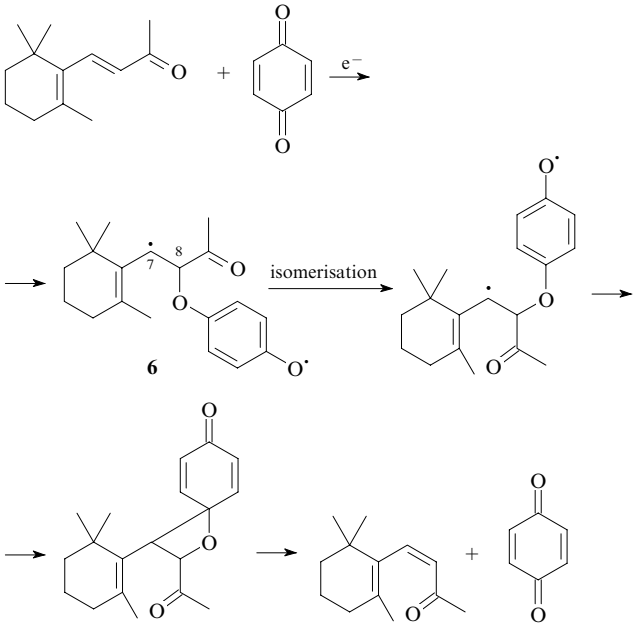


9,13-di-*cis*

3. *cis–trans* Isomerisation of C=C bonds in biradicals and neutral radicals

Due to the presence of many double bonds, carotenoids are potential reagents for the addition of ketones or aldehydes to alkenes (the Paterno–Büchi reaction) to form biradicals. Thus the formation of biradicals in the photoreaction of β-ionone with quinones was inferred from the results of CIDNP.[125] The formation of the polarised *cis*-isomer of β-ionone in the reaction with chloranil under the conditions of insufficient energy for the triplet recombination of the RIP points to the 'biradical' isomerisation route (see Scheme 2).

Isomerisation of β-ionone can occur as a result of rotation around the C(7)–C(8) bond in biradical **6**.



A similar mechanism for the *cis–trans* photoisomerisation of alkenes has been described earlier.[63, 64] As was noted above, once photoexcited, β-ionone can exhibit not only electron-donating, but also electron-withdrawing properties.[115, 125] Isomerisation of β-ionone in the photolysis in the presence of electron donors can proceed *via* either triplet β-ionone or a neutral radical, namely, the protonation product of the β-ionone radical anion. (It is known that the β-ionone radical anion undergoes fast protonation in the presence of hydrogen donors.[116]) According to results of CIDNP, the only isomerisation product of the neutral radical *trans* form is the corresponding *cis* isomer.[115]

Thus, the results accumulated to date make it possible to state that *cis–trans* isomerisation, which is the most important process in carotenoid chemistry, proceeds in the presence of electron acceptors or electron donors through the single-electron transfer stage.

VI. Inclusion complexes of carotenoids with cyclodextrins

As was mentioned above, the high reactivity, hydrophobicity and photosensitivity of carotenoids complicate their application; hence, in practice (food industry, cosmetology and medicine), their complexes with natural compounds are used. Inclusion complexes of carotenoids with cyclodextrins are studied most comprehensively. Cyclodextrins readily react with organic molecules to form inclusion complexes, which is explained by the peculiarities of their structure (the inner cavity of a cyclodextrin molecule is hydrophobic, while its outer surface is hydrophilic). Hydrophobic carotenoid molecules easily interact with the hydrophobic cavity. The complexes themselves are hydrophilic due to the hydrophilic outer surface of the cyclodextrin molecule. Numerous studies were devoted to the synthesis and properties of these complexes. In applied studies, attention was focused on the improvement of solubility of carotenoids and enhancement of their stability in aqueous solutions,[20–22, 25, 28, 134] while the fundamental studies considered the structure of complexes,[23, 24, 29] bioaccessibility of carotenoids at the cell level, penetration of carotenoids through cell membranes,[26, 27] antioxidant properties of complexes [29] and the use of cyclodextrins as a model of a natural enzyme responsible for β-carotene cleavage.[37, 135]

In food industry, carotenoids are used most widely as food pigments. The use of carotenoid complexes with cyclodextrins in place of free carotenoids allows one not only to obtain more stable colour of foodstuffs, but also to increase the storage life of food pigments themselves. Particularly, substitution of a cyclodextrin complex of astaxanthin for pure astaxanthin in the ration of salmon fish substantially increased the stability of salmon meat during storage.[134]

Recently, the problem of provision of humans with optimum amounts of vitamins becomes more pressing. Now, it is evident that this cannot be achieved with ordinary foodstuffs. A demand for the development of foodstuffs enriched with vitamins and biologically active additives has arisen. The most promising food additives include carotenoids, *e.g.*, β-carotene. A β-carotene complex with β-cyclodextrin (in Russia, it is produced under a name 'Tsiklokar') is widely used as a bioactive additive. Recently, it has been shown that the use of other cyclodextrins, *e.g.,* α-cyclodextrin and, especially, methylated β-cyclodextrins produces a greater effect;[28] however, in practice, the use of β-cyclodextrin rather than its derivatives are apparently associated with its availability and low cost.

In cosmetology, carotenoids are largely used as antioxidant additives in the production of protective creams. β-Carotene is used most often. However, its addition to make-up preparations results in their strong brick-red colour; this is why cosmetologists proposed to add not carotene as such, but its complexes, *e.g.*, a cyclodextrin complex of β-carotene. Its colour is much weaker § as compared with β-carotene solutions in organic solvents, and creams prepared with this additive have a nice rose colour.[22]

It should be noted that the applied studies did not discuss the problem of retention of useful properties of carotenoids upon their complexation, particularly, of their antioxidant activity. Insofar as the antioxidant activity of carotenoids is associated with their ability to scavenge free radicals, it can be assumed that its complexation would affect the latter process. Indeed, the ability of cyclodextrin to conserve, in its cavity, benzyl radical formed upon photolysis of an inclusion complex of cyclodextrin with dibenzyl ketone has been demostrated.[136]

The interaction of a water-soluble cyclodextrin complex of 8′-apo-β-caroten-8′-al with free peroxide radicals was studied.[29] The EPR spin-trapping method has shown that the complexation of 8′-apo-β-caroten-8′-al with β-cyclodextrin blocks almost completely the ability of this carotenoid to scavenge peroxide radicals. Apparently, this effect of cyclodextrin on the reactivity of carotenoids can be explained by the fact that the formation of complexes makes the cyclohexene ring of the carotenoid ¶ inaccessible for these radicals.[137] However, the increase in the carotenoid stability in complexes should not prevent their use *in vivo*, *e.g.*, for the delivery of carotenoids directly to the cell. Insofar as carotenoid complexes with cyclodextrins are not covalently bound rigid structures, but are in equilibrium with their starting components, it can be assumed that on the surface of a cell membrane, the complex will dissociate to release a carotenoid, which will penetrate into the membrane.

The role of cyclodextrins as the carriers of drugs, for example, carotenoids, was studied *in vitro* by many scientists (*e.g.*, see Refs 26, 27 and 138). In all the cases, the positive role of cyclodextrins in the delivery of carotenoids to cells was emphasised. Thus a comparison of such properties of β-carotene complexes with methylated β-cyclodextrin as bioaccessiblity, toxicity and stability with those of homogeneous solutions of β-carotene in a THF–DMSO solvent mixture has shown that the β-carotene complexes with cyclodextrin are, first, non-toxic, and, second, characterised by better bioaccessibility. For instance, after several days of incubation of cells with these complexes the level of β-carotene was substantially higher than that observed upon the use of homogeneous solutions of β-carotene.[27]

In conclusion, we consider yet another interesting application of cyclodextrins, *viz.*, the development of new enzymes, particularly, the enzyme responsible for splitting of β-carotene in organism. Thus an attempt was undertaken to model the action of the enzyme, namely, 15,15′-dioxygenase, which consists of the cleavage of the central C(15)–C(15′) bond of β-carotene to form two molecules of vitamin A.[37, 135] The goal was to obtain an unstable β-carotene intermediate, namely, the adduct of a H_2O_2 molecule to the central double bond of the carotenoid, which would readily decompose to afford two retinal molecules under the action of the synthetic enzyme.

The implementation of this idea involved the use of the Fenton reaction (7) where Fe^{2+} ions were replaced by a metalloporphyrin molecule. The enzyme model consisted of two β-cyclodextrin molecules covalently attached to the metalloporphyrin molecule. It was assumed that both cyclohexene rings of β-carotene will be captured by cyclodextrin molecules in such a way that the metal ion of the metalloporphyrin fragment will be located near the central double bond of the carotenoid. In this case, the reaction of H_2O_2 with the metal ion would proceed in the immediate vicinity of the central double bond of the carotenoid. Presumably, it is this double bond that would undergo the attack by the hydroxyl radical formed in the Fenton reaction. However, the use of the model iron porphirin-containing enzyme resulted in the formation of not only retinal, but also a wide set of other products, which suggests that oxygenation of different double bonds occurred.[135] At the same time, a synthetic enzyme containing ruthenium porphyrin did catalyse the cleavage of the central double bond of β-carotene.[37]

VII. Supramolecular complexes of carotenoids with glycyrrhizin

The revealed effect of complexation on the physical properties and reactivity of inclusion compounds initiated the search for new

§ Japanese authors also used the term 'colourless' to characterise this complex.[21]

¶ Peroxide radicals attack the terminal double bond of the cyclohexene fragment of a carotenoid.[29]

natural complexation agents that would exhibit certain advantages over cyclodextrins and, first of all, have no limitations with respect to the size of the 'guest' molecule. These compounds should form more stable complexes, reduce the toxic effect of drugs on the organism and, at the same time, enhance their therapeutic activity. Despite the complexity of this problem, one of such compounds has been found. This is glycyrrhizic acid (another popular name is glycyrrhizin), which is isolated from liquorice root (*e.g.*, of *Glycyrrhiza uralensis* Fish).[38] This compound has long been used in medicine as an anti-inflammatory agent or an expectorant. It is glycyrrhizin that provides the sugary taste to liquorice root.

In contrast to cyclodextrin, the glycyrrhizin molecule has an open-chain structure and contains both hydrophilic (glucuronic acid) and hydrophobic (glycyrrhetinic acid) parts. Glycyrrhizin has several important advantages over cyclodextrins. First, it belongs to open-type ligands, hence, its complexes have no rigorous restrictions for the size of the guest molecule. Second, its inclusion complexes are extraordinarily stable,[39–41] their stability constants exceed the mean stability constant of cyclodextrin complexes by two orders of magnitude.[30]

Glycyrrhizin is a component of many medicinal preparations. This is known to increase the therapeutic activity of drugs, which was attributed to their complexation with glycyrrhizin,[30,39] and to affect the reactivity of inclusion compounds.[42,139]

The above-mentioned aspects were studied by modern physicochemical methods by the example of carotenoid complexes[19,140] and two antiarrhythmic preparations, namely, Nifedipine and Lappaconitine.[42,139] The formation of inclusion complexes with glycyrrhizin was judged from the changes in the absorption and fluorescence spectra upon the addition of glycyrrhizin and from the changes in the reactivity of carotenoids.

1. Measurements of the stability and stoichiometry of carotenoid complexes with glycyrrhizin

The determination of stoichiometry and the measurements of stability constants of inclusion complexes formed upon mixing of carotenoids with glycyrrhizic acid were carried out using standard procedures (*e.g.*, see Refs 31, 141 and 142), *viz.,* Job's method and spectral methods, which allow determination of the stoichiometry and the stability constants of complexes, respectively, based on the dependence of the optical density of a solution on the GA concentration.

The structure, stability and reactivity of inclusion complexes of certain carotenoids with β-glycyrrhizic acid were studied.[140] The absorption spectra of carotenoids were shown to change with the GA concentration. The changes were observed only in aqueous solutions and their magnitude did not exceed 10%. It should be noted that the NMR method which is often used in the analysis of carotenoid complexes with cyclodextrins,[143] proved to give little information on these complexes. This is explained first of all by low solubility of carotenoids and GA in aqueous solutions and also by insufficient sensitivity of the NMR technique due to small concentrations of the components (10^{-4}–10^{-5} mol litre^{-1}).[143]

At the same time, fluorescence spectra of carotenoids complexes proved to be very sensitive to the formation of carotenoid complexes with glycyrrhizin (Fig. 5 *a*). Thus studies of the dependence of the intensity of fluorescence of canthaxantin on the glycyrrhizin concentration have shown that the stability of complexes at the GA concentration lower than 1 mmol litre^{-1} [$K = (0.3-1.0)\times10^4$ litre mol^{-1}, curve *1* in Fig. 5 *b*] differs substantially from that at the GA concentration larger than 1 mmol litre^{-1} ($K < 10^2$ litre mol^{-1}, curve *2* in Fig. 5 *b*). Earlier, Romanko and Murinov[144] have observed a sharp change in the dynamic viscosity of aqueous–ethanolic solutions (5%–10% ethanol) of glycyrrhizin at the GA concentration of ~1 mmol litre^{-1}. It was assumed that at this concentration glycyrrhizin forms micelle-like associates in aqueous solutions.†

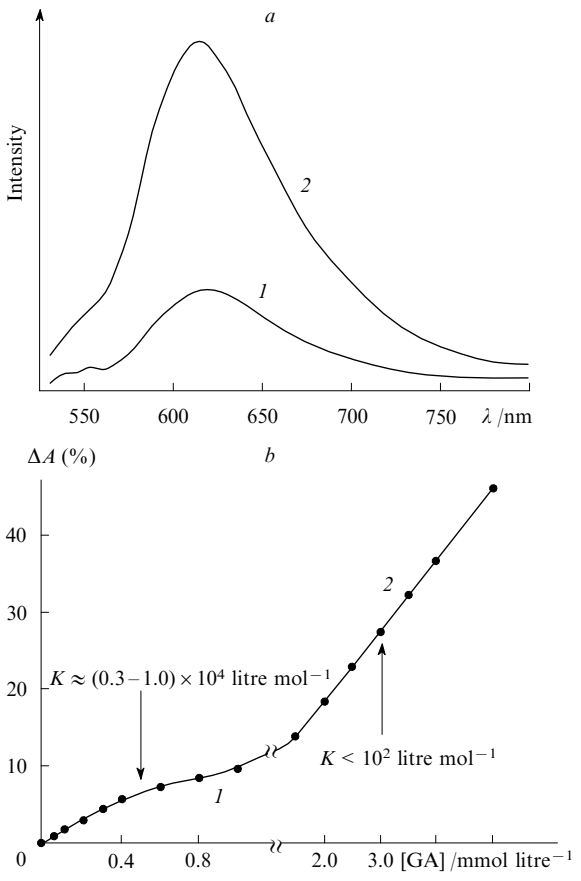


Figure 5. Fluorescence spectra of a canthaxanthin solution (0.02 mmol litre^{-1}) in the absence (curve *1*) and in the presence (curve *2*) of glycyrrhizic acid (5 mmol litre^{-1}) in DMSO containing 5% of water (*a*); changes in the fluorescence intensity (ΔA) of canthaxanthin as a function of the GA concentration in solution (*b*).[140]
Excitation wavelength was 470 nm, detection wavelength was 620 nm.

The stoichiometric ratios of components in associates were estimated based on the dependence of the changes in the optical density of the solution of carotenoid and glycyrrhizin (ΔD) on the carotenoid molar fraction r = [Car]/([Car] + [GA]) at a constant overall concentration of the components (Job's diagram, Fig. 6).[140] For example, the position of the maximum in the $\Delta D = f(r)$ curve at $r = 0.33$ corresponds to 1 : 2 ratio of carotenoid and glycyrrhizin molecules in the associate.

Earlier, it was supposed that GA molecules in aqueous solution form cylindrical dimers of the torus[40] or podand[41] types. It can be assumed that the hydrophobic interaction of carotenoids with glycyrrhizin will favour the penetration of the carotenoid molecule into the cyclic dimer formed. To check this hypothesis, the thermodynamic parameters (ΔG, ΔH and ΔS) of complexation of carotenoids with glycyrrhizin were measured.[140] Using standard relationships

$$-\Delta G = RT\ln K,$$

† The hypothesis of micelle formation was confirmed later by Japanese authors[145] who studied the formation of micelles of water-soluble derivatives of glycyrrhizin, particularly, Na salt of GA sulfate. We failed to find the data on the micelle formation in other solvents in the literature.

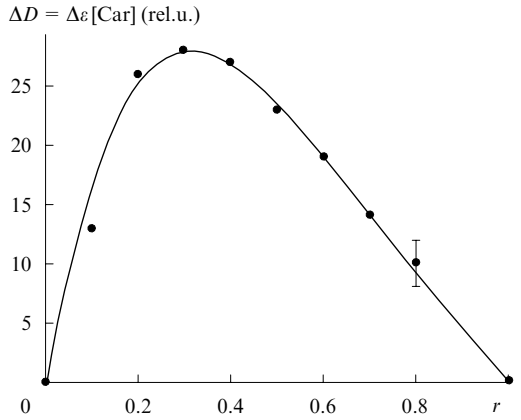


Figure 6. Job's diagram plotted based on the changes in the optical density of a solution in 20% ethanol at 433 nm as a function of the mole fraction of carotenoid (r);[140]
ε is the extinction coefficient.

$$\Delta G = \Delta H - T\Delta S$$

and

$$\ln\frac{K_2}{K_1} = -\frac{\Delta H}{R}\left(\frac{1}{T_2} - \frac{1}{T_1}\right)$$

(K_1 and K_2 are the stability constants of the complex at temperatures T_1 and T_2, respectively), the following values of parameters at $T = 293$ K were obtained: $\Delta H = 42.9$ kJ mol^{-1}, $\Delta G = -19.9$ kJ mol^{-1} and $\Delta S = 211$ J mol^{-1} K^{-1}. The positive values of the enthalpy and entropy terms suggest the preferential contribution of the hydrophobic interaction between the carotenoid and glycyrrhizin molecules into the stability of the complex formed and also substantial desolvation of the glycyrrhizin molecule, which is typical of inclusion complexes.

Inclusion complexes of carotenoids with GA of a composition 1 : 2 are formed both in aqueous solutions and in polar solvents such as DMSO, acetonitrile and alcohols. It is assumed that the complex represents a carotenoid molecule placed inside a torus formed by the dimer of glycyrrhizic acid.

Stability constants of complexes were estimated based on changes in the optical density of the solution at a fixed carotenoid concentration and variable glycyrrhizin concentration (Fig. 7). If the carotenoid concentration is much lower than that of glycyrrhizin, the stability constant of their complex can be calculated based on the Benesi – Hildebrand method.[142]

$$\frac{D}{\Delta D} - 1 = \frac{1}{K}\frac{1}{[\mathrm{GA}]\,[\mathrm{GA}]^n},$$

where $\Delta D = \Delta\varepsilon[\mathrm{Car}]$, K is the stability constant of the complex

$$K = \frac{[\mathrm{Car}-\mathrm{GA}_n]}{[\mathrm{Car}]\,[\mathrm{GA}]^n}. \qquad (8)$$

This allowed Polaykov *et al.*[140] not only to estimate the stability constant of complex (K), but also to determine the reaction order (n) of complex formation in a single experiment.

$$\mathrm{Car} + n\,\mathrm{GA} \overset{K}{\rightleftharpoons} \mathrm{Car}{-}\mathrm{GA}_n\,.$$

For complexes of the composition 1 : 2, a linear dependence between the absorption changes and the GA concentration was observed on the coordinates $1/\Delta D$ *vs.* 1/[GA]. The analysis of Job's diagram (see Fig. 6) showed that complexation of carotenoids with glycyrrhizin is a second-order reaction. Based on this, it was concluded that the complexation involves one carotenoid molecule and one glycyrrhizic acid dimer.

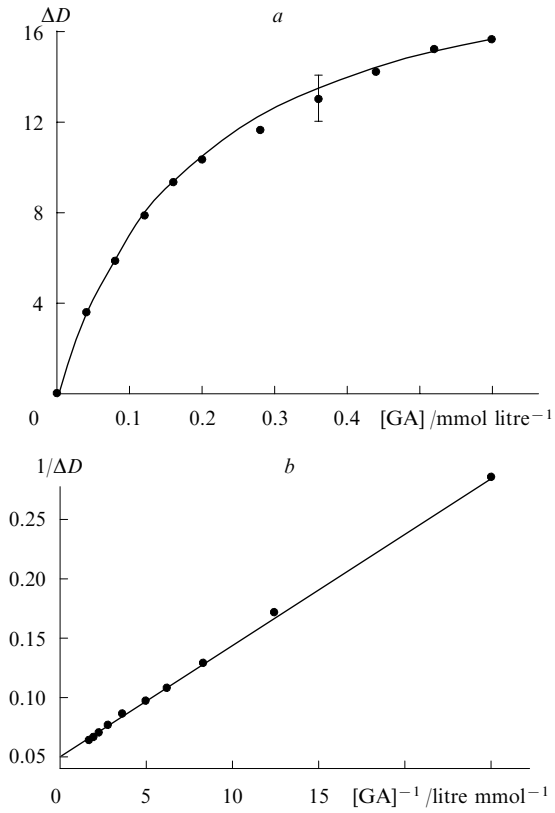


Figure 7. Changes in the optical density of a solution of β-carotene in 20% ethanol (2.5 μmol litre^{-1}) at 440 nm as a function of the concentration of glycyrrhizic acid (*a*) and the Benesi – Hildebrand diagram (*b*).[140] Points correspond to experimental data, solid lines correspond to calculations for a β-carotene – GA complex with a stability constant $K = 10^4$ litre mol^{-1}.

$$\mathrm{Car} + \mathrm{GA}_2 \overset{K}{\rightleftharpoons} \mathrm{Car}{-}\mathrm{GA}_2\,.$$

2. Reaction of carotenoids and their complexes with $FeCl_3$ in polar media

The data on the effect of glycyrrhizin on the reactivity of carotenoids are of undeniable interest. As was noted above, electron transfer reactions play the key role in the chemistry of carotenoids. It was found [140] that electron transfer in reactions of Fe(III) salts (electron acceptors) with complexes of carotenoids with glycyrrhizin was decelerated as compared with reactions of these salts with free carotenoids and the lifetime of carotenoid radical cations in glycyrrhizin complexes in polar media increased, which resulted in the change in the ratio of reaction products. Optical methods were used for studying the reactivities of inclusion complexes of carotenoids.[140] The decrease in the rate of direct electron transfer from carotenoids (β-carotene and 8′-apo-β-caroten-8′-al) involved in GA complexes to Fe^{3+} was inferred from a considerable (by a factor of several dozens) decrease in the absorption of carotenoid radical cations in the presence of GA as compared with their homogeneous solutions (Fig. 8), as well as from the rate of consumption of the carotenoids during the reaction.

It is known that the second oxidation potential of β-carotene coincides with its first potential;[146] this is why its reaction with $FeCl_3$ proceeds by different mechanisms for [Car] $\geqslant$ [Fe^{3+}] and for [Car] $\ll$ [Fe^{3+}]. In the former case, oxidation of β-carotene produces radical cation (Fig. 8 *a*) and in the latter case, the dication (Fig. 9 *a*). (The signal at 935 nm corresponds to the absorption of β-carotene radical cations, while the signal at 889 nm corresponds to the absorption of the dication.) When

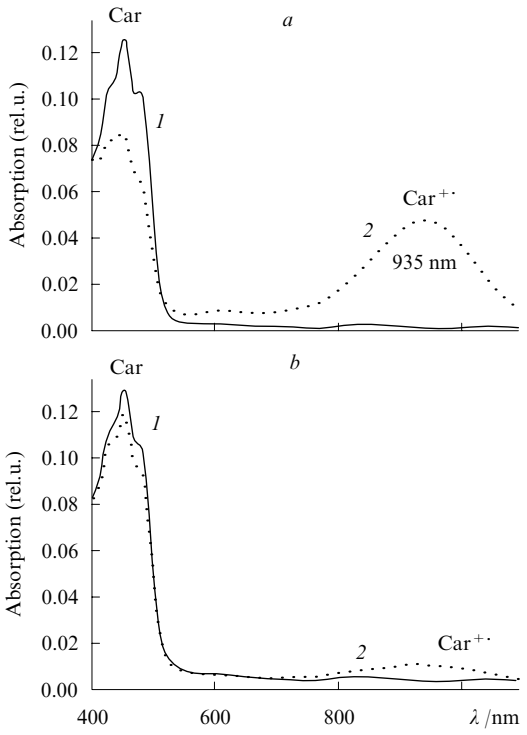


Figure 8. Absorption spectra of β-carotene recorded before (curve *1*) and after (curve *2*) mixing with the equivalent amount of $FeCl_3$ in acetonitrile at room temperature.[140]
(*a*) In the absence of glycyrrhizin; (*b*) in the presence of 0.1 mmol litre^{-1} of glycyrrhizin. [Car] = [$FeCl_3$] = 1.3 μmol litre^{-1}.

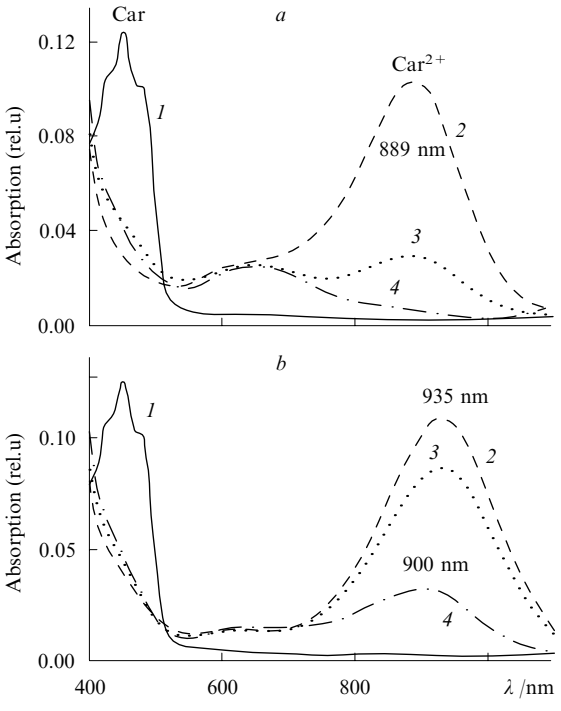


Figure 9. Absorption spectra of β-carotene recorded before (curve *1*) and after (curves *2*–*4*) mixing with an excess of $FeCl_3$ in acetonitrile at room temperature.[140]
(*a*) In the absence of glycyrrhizin; (*b*) in the presence of 0.1 mmol litre^{-1} of glycyrrhizin. [Car] = 1.3 μmol litre^{-1}, [$FeCl_3$] = 7 μmol litre^{-1}, (*1*) spectrum of β-carotene, (*2*) spectrum of a solution of β-carotene and $FeCl_3$ immediately after mixing, (*3* and *4*) spectra measured 6 and 20 min after the addition of $FeCl_3$, respectively.

β-carotene reacted with an excess of Fe^{3+} in the presence of GA, the stabilisation of the β-carotene radical cation was observed (Fig. 9 *b*).

Thus, the observed [140] differences in the reactions of Fe^{3+} salts with carotenoids either incorporated in glycyrrhizin complexes or in the free state suggest that the changes in the reactivity of carotenoids upon the addition of glycyrrhizin are caused by the formation of complexes. The concomitant increase in the lifetime of charged forms of carotenoids (cations and dications) by a factor of dozens implies that these species do not leave the complex being stabilised inside it.

3. Reactions of carotenoids and their complexes with quinones in polar media

The reactions of carotenoids with quinones is yet another example of the effect of complexation on their reactivity. Quinones were chosen because they are important natural electron acceptors.

The system β-carotene – 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (the latter was taken because its reaction with carotenoids does not require additional photo- or thermoactivation due to its low reduction potential) was chosen as the model system for studying the reactivity of inclusion compounds.[140] The formation of radical cations upon the addition of the quinone to a solution of β-carotene was judged from the changes in the absorption spectrum of the latter.

A characteristic feature of this reaction is that the curves that describe the decay kinetics of β-carotene radical cations incorporated in a glycyrrhizin complex or in solution have different shapes (Fig. 10). For a free carotenoid, the radical cation signal decays exponentially (the radical lifetime is ~ 20 s), whereas in the presence of glycyrrhizin, the signal decay curve demonstrates

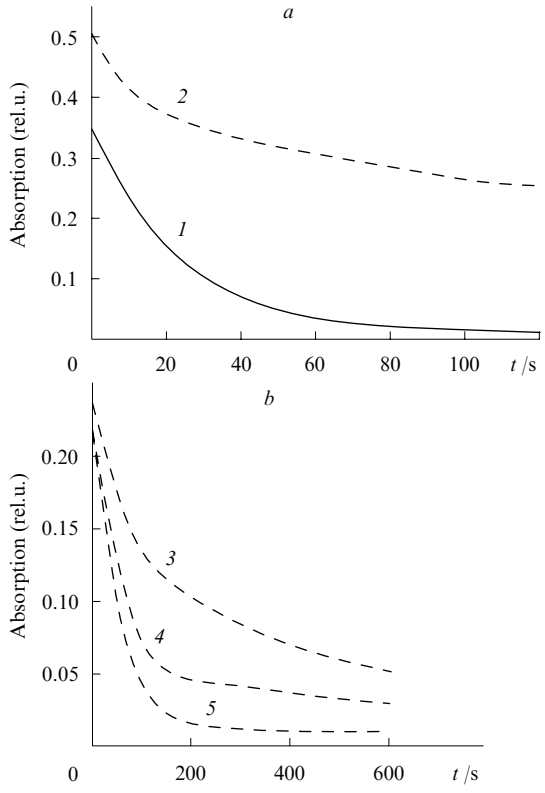


Figure 10. Decay kinetics of the absorption signal of β-carotene radical cation formed upon addition of DDQ to β-carotene in the absence (curve *1*) and in the presence of different amounts of glycyrrhizin (curves *2*–*5*).[140]
(*a*) [Car] = [DDQ] = 4 μmol litre^{-1}, (*b*) [Car] = 2 μmol litre^{-1}, [DDQ] = 5 μmol litre^{-1}; GA concentration /mmol litre^{-1}: 0.3 (*2*), 0.1 (*3*), 0.06 (*4*), 0.03 (*5*).

two distinct regions, which suggests the contribution of two processes, namely, a fast exponential and a slow ones (Fig. 10 *b*).

$$I = \frac{a}{1 + t/\tau}.$$

This two-component decay kinetics of the carotenoid radical cation made it possible to suggest that in solutions containing glycyrrhizin both the radical cations of the free carotenoid ($\tau \approx 20$ s) and the radical cations incorporated in the complex ($\tau \approx 1000$ s) are present. (Note that the contributions of these two states can be observed separately only in the absence of fast exchange between them.) The different shapes of decay curves at 935 nm for different glycyrrhizin concentrations (Fig. 10 *b*) point to the increase in the contribution of the slow component with the increase in the GA concentration, this contribution increasing proportionally to the square of GA concentration.[140] Such a square-law dependence could stem from the fact that the structure of the GA complex of the carotenoid radical cation in acetonitrile corresponds to the stoichiometry of 1 : 2. Bearing in mind that the contribution of the slow component is about 50% at the glycyrrhizin concentration of 0.1 mmol litre^{-1}, it can be concluded that for this concentration the ratio of free radical cations and their complexes is $\sim 1 : 1$. Based on expression (8), the stability constant of the carotenoid radical cation complex was estimated to be of an order of magnitude of 10^8 litre^2 mol^{-2}.

From the aforesaid, it follows that the stabilisation of the carotenoid radical cation due to its complexation with glycyrrhizin should substantially change the yield of reaction products of carotenoids with quinones. Thus if the CTC formed in the first stage is located inside the complex, the yield of the 'in-the-cage' reaction product, *i.e.*, the adduct of the carotenoid to the quinone, is increased. Otherwise, the increase in the lifetime of the free radical cation would increase the yield of carotenoid *cis*-isomers. Analysis of the composition of reaction products of certain carotenoids (β-carotene, canthaxathin and 7′-apo-7′,7′-dicyano-β-carotene) with DDQ in the presence and in the absence of glycyrrhizin using HPLC (Ref. 140) has shown that in all cases, the yield of carotenoid-to-quinone adducts substantially increased when the reaction was carried out in the presence of glycyrrhizin.

This experiment made it possible to draw an important conclusion that glycyrrhizin forms stable associates not only with individual compounds and their ions, but also with charge-transfer complexes; moreover, the complexation can result in substantial changes in the reaction route and the ratio of products.

4. Reactions of carotenoids and their complexes with free radicals

Ultimately, let us consider how the complexation of carotenoids with glycyrrhizin affects the antioxidant activity of carotenoids. This problem has recently been studied in detail.[19] The reaction rates of carotenoids with free radicals were estimated by EPR spin-trapping technique using *N*-*tert*-butyl-α-phenylnitrone as the spin trap.

It should be noted that when dealing with traps, it is very important to correctly identify the spin adducts observed.[19, 147, 148] Several test experiments were carried out to prove the correctness of assignment of the EPR signal observed in dimethyl sulfoxide to the adduct PBN−OOH. [14, 19] First, it was found that the shape of the EPR spectrum changed depending on the concentration of hydrogen peroxide in solution.[14] At low H_2O_2 concentrations ($[H_2O_2] = [FeCl_2] = 1$ mmol litre^{-1}), only the adduct PBN−CH_3 (DMSO was the origin of $^{\bullet}CH_3$) was formed, while at high concentrations ($[H_2O_2] = 500$ mmol litre^{-1}) the adduct PBN−OOH was formed and the adduct PBN−CH_3 disappeared as a result of the reaction of the $^{\bullet}CH_3$ radical with H_2O_2.[14]

The adducts of PBN with $^{\bullet}$OOR radicals are unstable, especially in the presence of transition metals that can reduce them to $^{\bullet}$OR.[147, 148] However, the majority of experimental facts that confirm this statement refer mainly to alkylperoxide radicals, whereas the adduct PBN−OOH was often observed under normal conditions.[149–151] The standard test for the adduct PBN−OOH is its reaction with superoxide dismutase. It was demonstrated [19] that the signal of the adduct PBN−OOH completely disappeared in the presence of this enzyme, whereas no changes in the spectrum of the adduct PBN−CH_3 occurred.

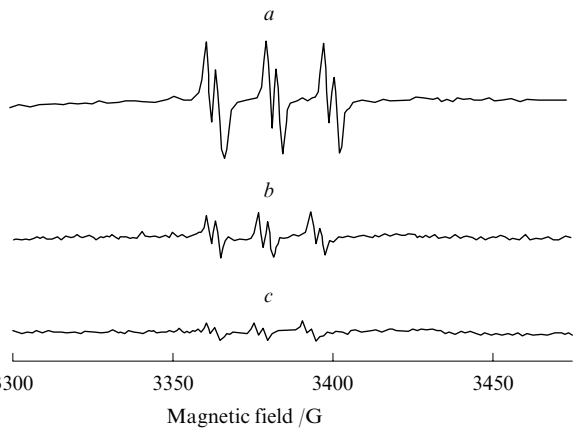


Figure 11. EPR spectra of the adduct PBN−OOH in the absence (*a*) and in the presence of canthaxanthin (*b*) and its complex with glycyrrhizin (*c*) in DMSO.[19] [PBN] = 5 mmol litre^{-1}, [$FeCl_2$] = 1 mmol litre^{-1}, [H_2O_2] = 0.5 mol litre^{-1}; [Car] = [GA] = 1 mmol litre^{-1}.

Moreover, a considerable decrease in the yield of the spin adduct PBN−OOH was observed in the presence of carotenoid complexes, which followed from the EPR spectra (Fig. 11).[19] Based on the experimental dependence of the yield of the spin product on the carotenoid concentration, the relative rate constant (k_{Car}/k_{ST}) for scavenging of the $^{\bullet}$OOH radical by a carotenoid was estimated according to Eqn (4). Note that one of the results of this study [19] was the observation of the antioxidant activity of glycyrrhizin as such. Therefore, the ratios k_{Car}/k_{ST} for different carotenoids were obtained by subtracting the contribution of glycyrrhizin to the overall rate constant for scavenging of the $^{\bullet}$OOH radical by a particular carotenoid (Table 1).

Table 1. Relative rate constant (k/k_{ST}) for scavenging of $^{\bullet}$OOH radicals by glycyrrhizic acid (GA) and GA complexes with zeaxanthin (ZX), canthaxanthin (CX) and 7′-apo-7′,7′-dicyano-β-carotene (DCC).[19]

[GA] /mmol litre^{-1}	k/k_{ST} [a]			
	GA	CX + GA	DDC + GA	ZX + GA
0	–	2	7	4
0.5	12	59	133	4
1	12	46	116	4
2	12	6	38	4

[a] Experimental error of k/k_{ST} determination is $\sim 10\%$.

Since the structures of carotenoid complexes with glycyrrhizin differ depending on whether the concentration of the latter is higher or lower than 1 mmol litre^{-1}, the rates of peroxide radical scavenging by free carotenoids and by their complexes in DMSO were compared for different concentrations of glycyrrhizin. The data listed in Table 1 show the presence of a synergistic effect, which manifests itself in the significant increase in the rate of scavenging of radicals by carotenoids [canthaxanthin (CX) and 7′-apo-7′,7′-dicyano-β-carotene (DCC)] incorporated in complexes with GA. The absence of the effect for zeaxanthin (ZX) was quite unexpected. The different behaviour of these three carotenoids was explained by their different polarographic half-wave oxidation potentials $E_{1/2}$. As is shown in Fig. 2, the k_{Car}/k_{ST}

vs. $E_{1/2}$ dependence is hyperbolic. A segment corresponding to the rapid changes in the rate of peroxide radical scavenging by a carotenoid is located close to $E_{1/2} = 0.7$ V. The oxidation potentials of canthaxanthin ($E_{1/2} = 0.68$ V) and 7′-apo-7′,7′-dicyano-β-carotene (0.72 V) fit exactly the region of 'fast changes' of scavenging rates, whereas the potential of zeaxanthin ($E_{1/2} = 0.56$ V) lies rather far in the plateau region. Bearing this in mind, the differences in the rates of radical scavenging by carotenoid complexes can be attributed to the changes in $E_{1/2}$ upon formation of the complexes. Indeed, even minor changes in the $E_{1/2}$ of CX and DCC can lead to a substantial increase in k_{Car}/k_{ST}.

To check this hypothesis, we studied the effect of complexation on the oxidation potentials of two carotenoids, namely, zeaxanthin and canthaxanthin.[19] Polarographic half-wave potentials were measured in 0.1 mmol litre^{-1} solutions of carotenoids in acetonitrile in the presence of 0.2 mmol litre^{-1} GA at room temperature. In both cases, E was observed to increase, *viz.*, by 0.05 V for CX and by 0.03 V for ZX. Such a change in the oxidation potential for zeaxanthin cannot cause any changes in its antioxidant activity, whereas a substantial increase in k_{Car}/k_{ST} will be observed for canthaxanthin. Yet another aspect of significance of this result is that it confirms the correctness of the earlier hypothesis on the role of electron transfer in scavenging of radical species by carotenoids.[14]

The results discussed above make it possible to conclude that complexation with glycyrrhizin has a substantial effect on the reactivity of carotenoids, *viz.*, this decreases the electron transfer rate in the reaction with electron acceptors and increases the lifetime of carotenoid radical cations in complexes, which results in changes in the ratio of reaction products of carotenoids with electron acceptors. Experimental results on the rates of scavenging of peroxide radicals by different carotenoids suggest that the changes in the oxidation potentials of carotenoids in complexes are among the key factors that determine their reactivity.

VIII. Conclusion

In the present review, the following homolytic reactions of carotenoids were considered: reactions with free radicals and complexation. The choice of these processes was caused by the keen interest of scientists in the antioxidant properties of carotenoids. As was mentioned above, the antioxidant activity of carotenoids is being extensively studied. Several reviews [14–17] on this subject have already been published; however, none of them provides the analysis of physicochemical factors responsible for this activity. Our review was intended to fill this gap.

We have demonstrated that electron transfer is typical of carotenoids not only in their transformations in photosynthetic centres or in the presence of electron donors or electron acceptors, but also in the reactions of carotenoids with free radicals and also in their *cis–trans* isomerisation. It can be stated that it is the tendency of carotenoids to single-electron transfer that unites the most significant biological processes involving carotenoids, from photosynthesis and photoisomerisation of retinal in visual pigment rhodopsin to their antioxidant activity.

The discussion, in this review, of carotenoid complexation processes was also associated with the studies of their antioxidant properties. At present, the development of effective pharmaceutical preparations based on inclusion complexes of natural compounds is one of the most rapidly developed fields of medicinal chemistry. However, insufficient attention is paid to the fundamental studies of the mechanism of complexation and physicochemical properties of inclusion complexes.

Particularly, the field of complexation of carotenoids with different 'host' molecules is still in its infancy. The information on the effect of complexation on the reactivity of 'guest' molecules clearly demonstrates the great prospects of this direction. In fundamental studies, these results make it possible to gain better insight into the role of weak interactions in complexation and also into the mechanism of the effect of organised media of the reactivity of inclusion compounds, while in applied studies, a possibility appears of the purposeful choice of complexation agents for strengthening (or weakening) of one or another property of carotenoids.

References

1. G Britton, S Liaaen-Jensen, H Pfander (Eds) *Carotenoids — Handbook* (Basel: Birkhauser, 2004)
2. L Packer, U Obermueller-Jevic, K Kraemer, H Sies (Eds) *Carotenoids and Retinoids: Molecular Aspects and Health Issues* (Champaign, IL: AOCS Press, 2005)
3. N I Krinsky, S T Mayne, H Sies (Eds) *Carotenoids in Health and Disease* (New York: Marcel Dekker, 2004)
4. T G Truscott *J. Photochem. Photobiol. B* **6** 359 (1990)
5. C E Cross, B Halliwell, E T Borish *Ann. Intern. Med.* **107** 526 (1987)
6. G W Burton, K U Ingold *Science* **224** 569 (1984)
7. T J Hill, E J Land, D J McGarvey, W Schalch, J H Tinkler, T G Truscott *J. Am. Chem. Soc.* **117** 8322 (1995)
8. S A Everett, M F Dennis, K B Patel, S Maddix, S C Kundu, R L Willson *J. Biol. Chem.* **271** 3988 (1996)
9. A Mortensen, L H Skibsted *Free Rad. Res.* **25** 515 (1996)
10. P F Conn, W Schalch, T G Truscott *J. Photochem. Photobiol., B* **11** 41 (1991)
11. P DiMascio, S Kaiser, H Sies *Arch. Biochem. Biophys.* **274** 532 (1989)
12. K H Helzlsouer, A J Alberg, E P Norkus, J S Morris, S C Hoffman, G W Comstock *J. Natl. Cancer Inst.* **88** 7 (1996)
13. K Jergensen, L H Skibsted *Z. Lebensm. Unters. Forsch.* **196** 423 (1993)
14. N E Polyakov, A I Kruppa, T V Leshina, T A Konovalova, L D Kispert *Free Rad. Biol. Med.* **31** 43 (2001)
15. S A R Paiva, R M Russell *J. Am. College Nutr.* **18** 426 (1999)
16. H D Martin, C Ruck, M Schmidt, S Sell, S Beutner, B Mayer, R Walsh *Pure Appl. Chem.* **71** 2253 (1999)
17. A El-Agamey, G M Lowe, D J McGarvey, A Mortensen, D M Phillip, T G Truscott, A J Young *Arch. Biochem. Biophys.* **430** 37 (2004)
18. T A Konovalova, L D Kispert, N E Polyakov, T V Leshina *Free Rad. Biol. Med.* **28** 1030 (2000)
19. N E Polyakov, T V Leshina, N F Salakhutdinov, T A Konovalova, L D Kispert *Free Rad. Biol. Med.* **40** 1804 (2006)
20. Jpn. P. 62267261; *Chem. Abstr.* **109** 110847 (1987)
21. Jpn. P. 04244059; *Chem. Abstr.* **118** 7320 (1992)
22. PCT WO 9513047; *Chem. Abstr.* **123** 122762 (1995)
23. A Mele, R Mendichi, A Selva *Carbohydr. Res.* **310** 261 (1998)
24. A Mele, R Mendichi, A Selva, P Molnar, G Toth *Carbohydr. Res.* **337** 1129 (2002)
25. L Szente, K Mikuni, H Hashimoto, J Szejtli *J. Inclusion Phenom. Mol. Recognit. Chem.* **32** 81 (1998)
26. I Lancrajan, H A Diehl, C Socaciu, M Engelke, M Zorn-Kruppa *Chem. Phys. Lipids* **112** 1 (2001)
27. I Pfitzner, P I Francz, H K Biesalski *Biochim. Biophys. Acta–Gen. Subj.* **1474** 163 (2000)
28. S M O Lyng, M Passos, J D Fontana *Proc. Biochem.* **40** 865 (2005)
29. N E Polyakov, T V Leshina, T A Konovalova, E O Hand, L D Kispert *Free Rad. Biol. Med.* **36** 872 (2004)
30. K A Connors *Chem. Rev.* **97** 1325 (1997)
31. J Szejtli, T Osa *Comprehensive Supramolecular Chemistry (Cyclodextrins)* Vol. 3 (Oxford: Elsevier, 1996)
32. I Miyazawa, H Ueda, H Nagase, T Endo, S Kobayashi, T Nagai *Eur. J. Pharm. Sci.* **3** 153 (1995)
33. T Loftsson, M E Brewster *J. Pharm. Sci.* **85** 1017 (1996)
34. H-J Buschmann, E Schollmayer *J. Cosmet. Sci.* **53** 185 (2002)
35. J Szejtli *Cyclodextrin Technology* (Dordrecht: Kluwer Academic, 1988)
36. F Bressolle, M Audran, T-N Pham, J-J Vallon *J. Chromatogr. B* **687** 303 (1996)

37. R R Franch, P Holzer, M Leuenberger, M C Nold, W-D Woggon *J. Inorg. Biochem.* **88** 295 (2002)
38. G A Tolstikov, L A Baltina, E E Shul'ts, A G Pokrovskii *Bioorg. Khim.* **23** 691 (1997) [a]
39. V N Maistrenko, V N Gusakov, Yu I Rusakov, Yu I Murinov, G A Tolstikov *Dokl. Akad. Nauk* **335** 329 (1994) [b]
40. E Yu Sangalov *Zh. Obshch. Khim.* **69** 667 (1999) [c]
41. V N Gusakov, V N Maistrenko, P P Safiullin *Zh. Obshch. Khim.* **71** 1382 (2001) [c]
42. N E Polyakov, V K Khan, M B Taraban, T V Leshina, T G Tolstikova, N F Salakhutdinov, G A Tolstikov, in *The 2nd International Conference on Natural Products and Physiologically Active Substances (Abstracts), Novosibirsk, 2004* p. 71
43. L D Kispert, T A Konovalova, Y Gao *Arch. Biochim. Biophys.* **430** 49 (2004)
44. C C Schenck, B Diner, P Mathis, K Satoh *Biochim. Biophys. Acta* **680** 216 (1982)
45. P Mathis, A W Rutherford *Biochim. Biophys. Acta* **767** 217 (1984)
46. J De Las Rivas, A Telfer, J Barber *Biochim. Biophys. Acta* **1142** 155 (1993)
47. D Kuciauskas, P A Liddell, A L Moore, T A Moore, D Gust *J. Am. Chem. Soc.* **120** 10880 (1998)
48. J Xiang, F S Rondonuwu, Y Kakitani, R Fujii, Y Watanabe, Y Koyama *J. Phys. Chem. B* **109** 17066 (2005)
49. X Wang, R Fujii, S Ito, Y Koyama, Y Yamano, M Ito, T Kitamura, S Yanagida *Chem. Phys. Lett.* **416** 1 (2005)
50. A Telfer, J De Las Rivas, J Barber *Biochim. Biophys. Acta* **1060** 106 (1991)
51. J Hanley, Y Deligiannakis, A Paskal, P Faller, A W Rutherford *Biochemistry* **38** 8189 (1999)
52. Y Deligiannakis, J Hanley, A W Rutherford *J. Am. Chem. Soc.* **122** 400 (2000)
53. J R Harbour, G Tollin *Photochem. Photobiol.* **19** 163 (1974)
54. G Feher, M Y Okamura, J D McElroy *Biochim. Biophys. Acta* **267** 222 (1972)
55. A van der Est, I Sieckmann, W Lubitz, D Stehlik *Chem Phys.* **194** 349 (1995)
56. D Kuciauskas, P A Liddel, S-C Hung, S Lin, S Stone, G R Seely, A L Moore, T A Moore, D Gust *J. Phys. Chem. B* **101** 429 (1997)
57. P Seta, E Bienvenue, A L Moore, P Mathis, R V Bensasson, P Liddell, P J Pessiki, T A Moore, D Gust *Nature (London)* **316** 653 (1985)
58. P A Liddell, D Kuciauskas, J P Simuda, B Nash, D Nguyen, A L Moore, T A Moore, D Gust *J. Am. Chem. Soc.* **119** 1400 (1997)
59. N E Polyakov, V V Konovalov, T V Leshina, O A Luzina, N F Salakhutdinov, T A Konovalova, L D Kispert *J. Photochem. Photobiol., A* **141** 117 (2001)
60. J L Grant, V J Kramer, R Ding, L D Kispert *J. Am. Chem. Soc.* **110** 2151 (1988)
61. E Paternò, C Chieffi *Gazz. Chim. Ital.* **39** 341 (1909)
62. G Büchi, C G Inman, E S Lipinsky *J. Am. Chem. Soc.* **76** 4327 (1954)
63. G Eckert, M Goez *J. Am. Chem. Soc.* **116** 11999 (1994)
64. M Goez, G Eckert *J. Am. Chem. Soc.* **118** 140 (1996)
65. Y L Gao, L D Kispert *J. Phys. Chem. B* **107** 5333 (2003)
66. Y L Gao, T A Konovalova, T Xu, L D Kispert *J. Phys. Chem. B* **106** 10808 (2002)
67. T A Konovalova, Y L Gao, L D Kispert, J van Tol, L-C Brunel *J. Phys. Chem. B* **107** 1006 (2003)
68. Y L Gao, L D Kispert, T A Konovalova, J N Lawrence *J. Phys. Chem. B* **108** 9456 (2004)
69. P Hapiot, L D Kispert, V V Konovalov, J-M Savéant *J. Am. Chem. Soc.* **123** 6669 (2001)
70. Y L Gao, T A Konovalova, J N Lawrence, M A Smitha, J Nunley, R Schad, L D Kispert *J. Phys. Chem. B* **107** 2459 (2003)
71. T G Truscott *J. Photochem. Photobiol., B* **35** 233 (1996)
72. H W-S Chan, in *Autoxidation of Unsaturated Lipids.* (Ed. H W-S Chan) (London: Academic Press, 1987) p. 293
73. P Palozza, N I Krinsky *Methods Enzymol.* **213** 403 (1992)
74. P Palozza, N I Krinsky *Arch. Biochem. Biophys.* **297** 291 (1992)
75. P Palozza, N I Krinsky *Free Rad. Biol. Med.* **11** 407 (1991)
76. A A Woodall, S W-M Lee, R J Weesie, M J Jackson, G Britton *Biochim. Biophys. Acta – Gen. Subj.* **1336** 33 (1997)
77. A A Woodall, G Britton, M J Jackson *Biochim. Biophys. Acta – Gen. Subj.* **1336** 575 (1997)
78. A Mortensen, L H Skibsted, J Sampson, C Rice-Evans, S A Everett *FEBS Lett.* **418** 91 (1997)
79. A J Young, G M Lowe *Arch. Biochem. Biophys.* **385** 20 (2001)
80. A Mortensen *J. Photochem. Photobiol., B* **61** 62 (2001)
81. A Mortensen *Free Rad. Res.*, **36** 211 (2002)
82. R C Mordi, J C Walton, G W Burton, L Hughes, K U Ingold, D A Lindsay, D J Moffatt *Tetrahedron* **49** 911 (1993)
83. R C Mordi, J C Walton, G W Burton, L Hughes, K U Ingold, D A Lindsay *Tetrahedron Lett.* **32** 4203 (1991)
84. D C Liebler, T D McClure *Chem. Res. Toxicol.* **9** 8 (1996)
85. D L Baker, E S Krol, N Jacobsen, D C Liebler *Chem. Res. Toxicol.* **12** 535 (1999)
86. A Mortensen, L H Skibsted *Free Rad. Res.* **25** 355 (1996)
87. A Mortensen, L H Skibsted *J. Agric. Food Chem.* **45** 2970 (1997)
88. A S Jeevarajan, L D Kispert, X Wu *Chem. Phys. Lett.* **219** 427 (1994)
89. J A Jeevarajan, C C Wei, A S Jeevarajan, L D Kispert *J. Phys. Chem.* **100** 5637 (1996)
90. A Mortensen, L H Skibsted *Free Rad. Res.* **26** 549 (1997)
91. A El-Agamey, D J McGarvey *J. Am. Chem. Soc.* **125** 3330 (2003)
92. G Britton *FASEB J.* **9** 1551 (1995)
93. W Miki *Pure Appl. Chem.* **63** 141 (1991)
94. ATBC (The α-Tocopherol, β-Carotene Cancer Prevention Study Group) *New Engl. J. Med.* **330** 1029 (1994)
95. G S Omenn, G E Goodman, M D Thornquist, J Balmes, M R Cullen, A Glass, J P Keogh, F L Meyskens, B Valanis, J H Williams, S Barnhart, S Hammar *New Engl. J. Med.* **334** 1150 (1996)
96. C H Hennekens, J E Buring, J E Manson, M Stampfer, B Rosner, N R Cook, C Belanger, F La Motte, J M Gaziano, P M Ridker, W Willett, R Peto *New Engl. J. Med.* **334** 1145 (1996)
97. G Block, B Patterson, A Subar *Nutr. Cancer* **18** 1 (1992)
98. E Giovannucci, A Ascherio, E B Rimm, M J Stampfer, G A Colditz, W C Willett *J. Natl. Cancer Inst.* **87** 1767 (1995)
99. W Stahl, H Sies *Arch. Biochem. Biophys.* **336** 1 (1996)
100. G van Poppel *Eur. J. Clin. Nutr.* **50** 57 (1996)
101. H S Black *Front Biosci.* 1044 (2002)
102. P Palozza *Nutr. Rev.* **56** 257 (1998)
103. P Palozza, G Calviello, S Serini, N Maggiano, P Lanza, F O Ranelletti, G M Bartoli *Free Rad. Biol. Med.* **30** 1000 (2001)
104. P Palozza, G Calviello, G M Bartoli *Free Rad. Biol. Med.* **19** 887 (1995)
105. N E Polyakov, T V Leshina, T A Konovalova, L D Kispert *Free Rad. Biol. Med.* **31** 398 (2001)
106. H Shi, L J Hudson *Free Rad. Biol. Med.* **37** 582 (2004)
107. D Galaris, A Evangelou *Crit. Rev. Oncol. Hematol.* **42** 93 (2002)
108. B Halliwell, M C Gutteridge *Methods Enzymol.* **186** 1 (1990)
109. H A Frank, A J Young, G Britton, R J Cogdell *The Photochemistry of Carotenoids* (Dordrecht: Kluwer Academic, 1999)
110. H Cerfontain, J A J Geenevasen *Tetrahedron* 1571 (1981)
111. J Milanowska, W I Gruszecki *J. Photochem. Photobiol., B* **80** 178 (2005)
112. M Kuki, Y Koyama, H Nagae *J. Phys. Chem.* **95** 7171 (1991)
113. N-H Jensen, R Wilbrandt, R V Bensasson *J. Am. Chem. Soc.* **111** 7877 (1989)
114. N E Polyakov, T V Leshina, L D Kispert *RIKEN Rev.* **44** 140 (2002)
115. N E Polyakov, T V Leshina *Mol. Phys.* **100** 1297 (2002)
116. K Bobrowski, P K Das *J. Phys. Chem.* **91** 1210 (1987)
117. K Nakanishi *Pure Appl. Chem.* **63** 161 (1991)
118. R R Rando *Angew. Chem., Int. Ed. Engl.* **29** 461 (1990)
119. Y Koyama *J. Photochem. Photobiol., B* **9** 265 (1991)
120. K M Salikhov, Yu N Molin, R Z Sagdeev, A L Buchachenko *Spin Polarization and Magnetic Effects in Radical Reactions* (Budapest: Academiai Kiadó, 1984)
121. T V Leshina, K M Salikhov, R Z Sagdeev, S G Belyaeva, V I Maryasova, P A Purtov, Yu N Molin *Chem. Phys. Lett.* **70** 228 (1980)
122. H D Roth, M L M Schilling *J. Am. Chem. Soc.* **101** 1898 (1979)

123. H G O Becker *Introduction to the Photochemistry* (Berlin: VEB Deutscher Verlag der Wissenschaften, 1976)
124. N E Polyakov, V S Bashurova, P S Schastnev, T V Leshina *J. Photochem. Photobiol., A* **107** 55 (1997)
125. N E Polyakov, A I Kruppa, V S Bashurova, R N Musin, T V Leshina, E Hand, L D Kispert *J. Photochem. Photobiol., A* **128** 65 (1999)
126. H Cerfontain, J A J Geenevasen, P C M van Noort *J. Chem. Soc., Perkin Trans 2* 1057 (1980)
127. A I Kruppa, T V Leshina, V V Konovalov, L D Kispert *J. Chem. Phys.* **103** 1414 (1999)
128. A I Kruppa, O I Mikhailovskaya, T V Leshina *Chem. Phys. Lett.* **147** 65 (1988)
129. F D Lewis, J R Petisce, J D Oxman, M J Nepras *J. Am. Chem. Soc.* **107** 203 (1985)
130. G Gao, C-C Wei, A S Jeevarajan, L D Kispert *J. Phys. Chem.* **100** 5362 (1996)
131. C-C Wei, G Gao, L D Kispert *J. Chem. Soc., Perkin Trans. 2* 783 (1997)
132. M Khaled, A Hadjipetrou, L D Kispert *J. Phys. Chem.* **94** 5164 (1990)
133. H Hashimoto, Y Koyama *J. Phys. Chem.* **92** 2101 (1988)
134. PCT WO 2004005353; *Chem. Abstr.* **140** 110455 (2004)
135. W-D Woggon *Chimia* **54** 564 (2000)
136. S S Petrova, A I Kruppa, T V Leshina *Chem. Phys. Lett.* **385** 40 (2004)
137. N E Polyakov, T V Leshina, E O Hand, A Petrenko, L D Kispert *J. Photochem. Photobiol., A* **161** 261 (2004)
138. S Arikan, R G Rodway *Animal Repr. Sci.* **64** 149 (2000)
139. N E Polyakov, V K Khan, M B Taraban, T V Leshina, N F Salakhutdinov, G A Tolstikov *J. Phys. Chem. B* **109** 24526 (2005)
140. N E Polyakov, T V Leshina, N F Salakhutdinov, L D Kispert *J. Phys. Chem. B* **110** 6991 (2006)
141. L Martín, A León, A I Olives, B del Castillo, M A Martín *Talanta* **60** 493 (2003)
142. E A Lopez, J M Bosque-Sendra, L C Rodriguez, A M G Campana, J J Aaron *Anal. Bioanal. Chem.* **375** 414 (2003)
143. L Fielding *Tetrahedron* **56** 6151 (2000)
144. T V Romanko, Yu I Murinov *Zh. Fiz. Khim.* **75** 1601 (2001) [d]
145. S Saito, T Furumoto, M Ochiai, A Hosono, H Hoshino, U Haraguchi, R Ikeda, N Shimada *Eur. J. Med. Chem.* **31** 365 (1996)
146. D Liu, L D Kispert, in *Recent Research Developments in Electrochemistry* (Ed. S G Pandalai) (Trivandrum, India: Transworld Research Network, 1999. P. 139
147. G R Buetter, in *Handbook of Methods of Oxygen Radical Research* (Ed. R A Greenwald) (Boca Raton, FL: CRC Press, 1986) p. 151
148. S I Dicalov, R P Mason *Free Rad. Biol. Med.* **27** 864 (1999)
149. G R Buettner *Free Rad. Biol. Med.* **3** 259 (1987)
150. J R Harbour, V Chow, J R Bolton *Can. J. Chem.* **52** 3549 (1974)
151. Y Yoshimura, T Inomata, H Nakazawa *J. Liquid Chromatogr. Relat. Technol.* **22** 419 (1999)

[a] — *Russ. J. Bioorg. Chem. (Engl. Transl.)*
[b] — *Dokl. Chem. (Engl. Transl.)*
[c] — *Russ. J. Gen. Chem. (Engl. Transl.)*
[d] — *Russ. J. Phys. Chem. (Engl. Transl.)*