Effect of carbonyls on the reactivity of polyenes in autoxidation

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ABSTRACT: Autoxidation of the biologically active polyenes β-carotene, canthaxanthin, retinyl acetate, methyl retinoate, retinal and 3,7,11,11-tetramethyl-10,15-dioxo-2,4,6,8-hexadecatetraenal (diketoretinal) in solid amorphous films was investigated. The course of the process was followed by electronic and IR spectroscopy. The overall activation energies were obtained. It was shown that insertion in the polyene molecule of a carbonyl group conjugated with polyene chain results in a drastic decrease in the reactivity towards molecular oxygen. The results are discussed and explained on the basis of radical stabilization energy, $E_S(R^{\cdot})$, as a driving force of the autoxidation process. Semi-empirical AM1 calculations of ΔH_f of some retinyl polyenes and polyenyl radicals were carried out and the C—H bond strengths and $E_S(R^{\cdot})$ values of corresponding polyenyl radicals were evaluated. It was shown that the incorporation of a carbonyl group in the polyene results in a decrease in the overall autoxidation rate due to the decrease in both the initiation and propagation rates. Copyright © 2003 John Wiley & Sons, Ltd.

KEYWORDS: autoxidation; polyenes; reactivity; carbonyls; radical stabilization energy; rate constants

INTRODUCTION

Retinyl polyenes and carotenoids, biologically active natural and synthetic compounds, are susceptible to oxidation by molecular oxygen (autoxidation). Numerous investigations have been devoted to elucidating the factors that influence the reactivity of polyenes towards oxygen. It was shown previously that the reactivity of the polyenic compounds studied exceeds the reactivity of other compounds by orders of magnitude.[1,2]

In this connection, it was considered of interest to establish the dependence of the reactivity of polyenes in autoxidation on the presence of functional groups. In this paper, we report the effect of carbonyls on the autoxidation of polyenes with various conjugated chain lengths.

EXPERIMENTAL

Materials and methods

The polyenes studied are shown in Scheme 1. The experimental procedures for the preparation and purification of the polyenes, excluding diketoretinal (**6**, Scheme

1), were described previously.[1,2] Diketoretinal (3,7,11,11-tetramethyl-10,15-dioxo-2,4,6,8-hexadecatetraenal) (**6**) was prepared by oxidation of retinal with
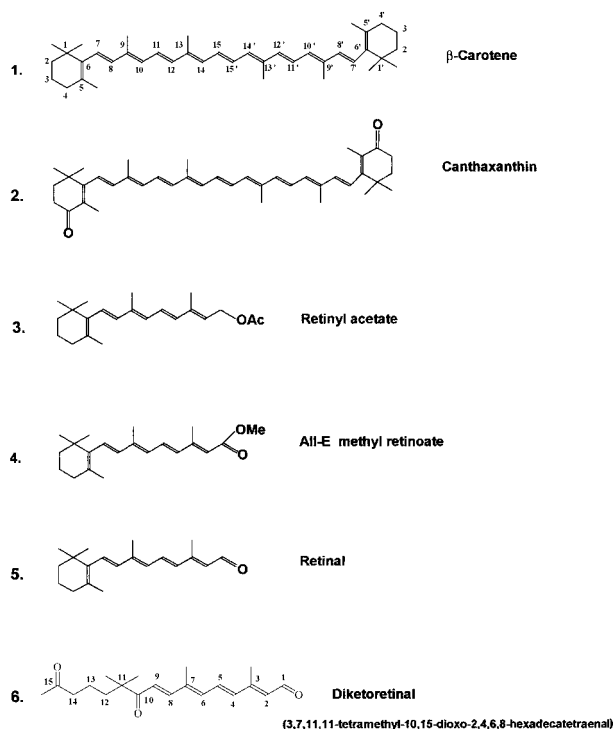
Scheme 1

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pyridinium chlorochromate in CH_2Cl_2. After filtration, dissolution of the residue in diethyl ether and removal of the solvent under reduced pressure, **6** was separated from other products by column chromatography on SiO_2 with hexane–diethyl ether as eluent. By crystallization from diethyl ether–hexane, yellow–brown crystals were obtained. UV, 369 nm (ε 58 000 $\pm$ 500); IR (in CCl_4), 1720, 1668 (C=O), 1596 (C=C), 984, 964 ($\delta_{=C-H}$) cm^{-1}. Literature values:[3] UV, 370 nm (ε 58395); IR, 1720, 1670, 1595, 970 cm^{-1}.

The purity of the carotenoids and retinyl polyenes was checked by high-performance liquid chromatography and was shown to be not less than 99%.

Special precautions were taken to avoid uncontrolled autoxidation. All the polyenes were stored *in vacuo* (10^{-2}–10^{-3} Torr) (1 Torr = 133.3 Pa) at about $-18\,°C$. The solvents (benzene, hexane, $CHCl_3$, CCl_4) were purified by standard techniques and dried over sodium metal and calcium hydride (benzene and hexane) and Al_2O_3 ($CHCl_3$ and CCl_4). After degassing, the dry solvents were vacuum condensed in glass tubes. The tubes were sealed at about 10^{-3} Torr. The solvents were saturated with argon before use by breaking the tubes in an argon atmosphere. The samples for autoxidation were solid amorphous films.

The amorphous films of the polyenes were prepared in air or a dry argon atmosphere (in an argon box, if necessary) by evaporation of one (or two) drops of the solution of a polyene on fast rotating quartz (for electronic spectra recording) or KRS-5 (for IR spectra recording) supports. The thickness of the films was estimated to be about 0.1 µm.

Electronic and IR spectral techniques were used to investigate the kinetics of autoxidation. The high extinction of the main absorption bands of the polyenes in the electronic spectra provides a convenient and precise way for the investigation of the autoxidation process. Thermostated quartz cells were used for recording electronic spectra. A support with the film was placed in the cell under an argon atmosphere (in an argon box). The cell was set in the sample compartment of the spectrophotometer and connected to a vacuum line and a manostat for oxygen feeding. After evacuation of the cell, the spectra of the film were recorded *in vacuo* and then in an oxygen atmosphere at a given oxygen pressure and temperature. In some cases autoxidation was performed in air at room temperature.

The IR spectra of the films were recorded by means of the multiple attenuated total reflection (ATR) technique. The spectra were obtained using an ATR unit with KRS-5 reflection elements ($49 \times 25 \times 3.5$ mm, 14 reflections, 45° facets). The ATR IR spectra were recorded by exposing the films to air in the spectrometer sample compartment.

The electronic spectra were recorded on a Specord M40 spectrophotometer and IR spectra on a Specord M82 spectrophotometer (Carl Zeiss, Jena, Germany).
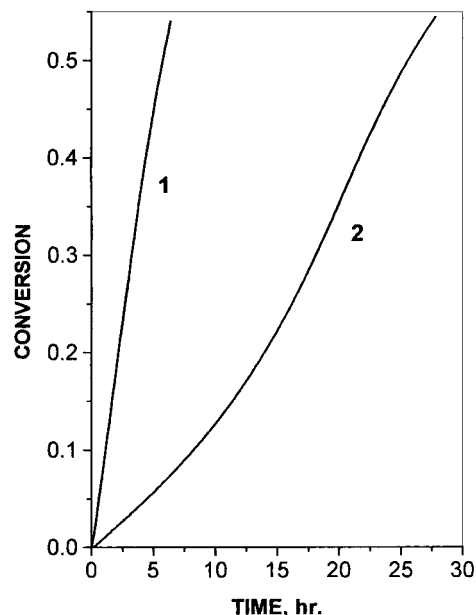

Figure 1. Kinetics of autoxidation of carotenoid films at room temperature: (1) β-carotene; (2) canthaxanthin

The conversion of the polyenes during the autoxidation was determined by monitoring the decrease in the optical densities of the absorption bands of the electronic spectra.

Computational methods

Semi-empirical computations of the heats of formation of some polyenes and corresponding radicals were performed by using the HYPERCHEM package (version 6.01) (Hypercube). The initial configurations of the different polyene molecules and radicals were build by standard geometric parameters and first optimized with the molecular mechanics MM+ program. Then the geometry optimization was achieved with AM1 calculations. By these calculations, energy minimization was obtained by lowering the gradient to ≤ 0.004 kcal $\mathring{A}^{-1}$ mol^{-1} (1 kcal = 4.184 kJ).

RESULTS AND DISCUSSION

The results obtained show the strong influence of carbonyl groups on the reactivity of polyenes towards molecular oxygen.

It is obvious that carbonyl groups conjugated to the polyene chain strongly reduce the oxidizability of polyenes. This conclusion follows from the comparison of the data for β-carotene and canthaxanthin (Fig. 1), retinyl acetate and methyl retinoate (Fig. 2), retinal and diketoretinal (Fig. 3). The overall activation energies of polyene autoxidation in solid films found previously[2] and in this work are presented in Table 1. The data show that the activation energy of autoxidation more than doubles
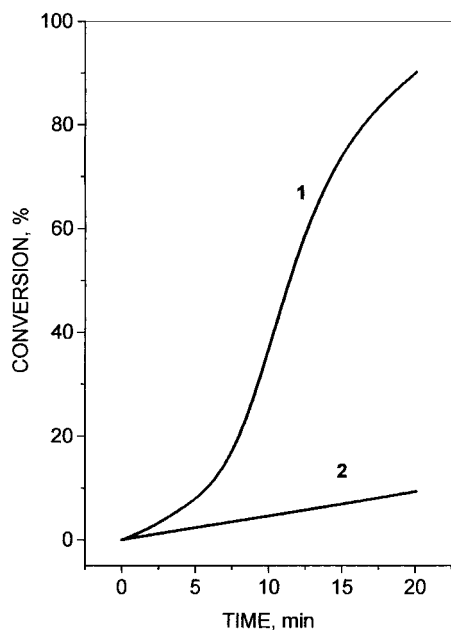
Figure 2. Kinetics of autoxidation of retinyl polyenes in solid films: (1) retinyl acetate at 20 °C and oxygen pressure 100 Torr; (2) methyl retinoate in oxygen atmosphere at 58 °C

Table 1. Activation energies for autoxidation of polyenes

Polyene	$T \Delta$ (°C)	E (kJ mol^{-1})
1	28–45	77 ± 3
2	35–45	160 ± 14
4	48–60	148 ± 3
5	25–45	42 ± 10
6	40–60	100 ± 16

autoxidation than the corresponding hydrocarbons (β-carotene and canthaxanthin, retinyl acetate and retinal).

The change in reactivity of the investigated retinyl polyenes may be explained at a qualitative level in terms of the energy of the highest bonding and lowest antibonding orbitals. Figure 4 shows the HOMO and LUMO levels of four retinyl polyenes with different oxygen substituents. A decrease in the energy of the HOMO and LUMO orbitals of the polyenes is observed in the following sequence: retinyl acetate >retinal >methyl retinoate >diketoretinal. The decrease is related to the increase in the nucleophilic properties of the molecules. The reactivity of a molecule towards the electrophilic radicals (RO$_2\cdot$) decreases with increase in nucleophilicity. Although this approach is qualitative, the trend is in agreement with the experimental data.

As was shown previously,[1] polyenes with 'allylic' hydrogen(s) are more reactive in autoxidation than polyenes that have no such reactive sites. Comparison of the data on the reactivity of β-carotene and canthaxanthin (Fig. 1) and retinal and diketoretinal (Fig. 3) suggests that the insertion of a second carbonyl at the end of the polyene chain removes a possible reactive site, diminishing the overall reactivity.

During the kinetic investigation of the autoxidation of retinyl polyenes, it was also found that the compounds of

due to the carbonyl conjugated to the polyene chain. This effect is opposite to the influence of carbonyl groups on the autoxidation rates of saturated compounds. It is well known that saturated ketones and aldehydes undergo autoxidation with much higher rates than those for the corresponding hydrocarbons under the same conditions[4,5]. The polyenic aldehydes and ketones of the retinol and carotene series, instead, undergo slower
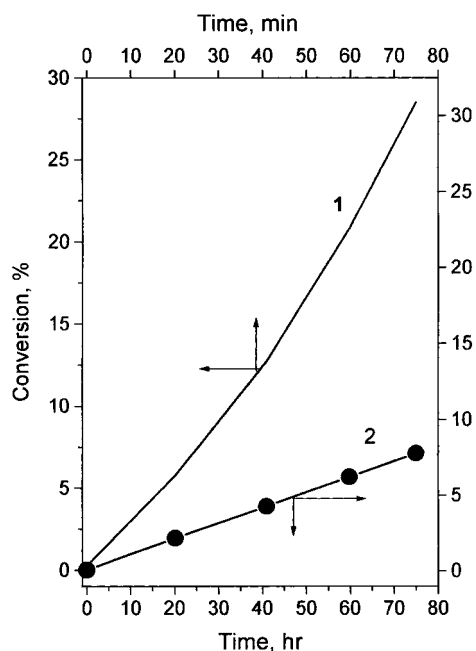

Figure 3. Autoxidation of polyene ketones in air at 25 °C: (1) retinal; (2) diketoretinal
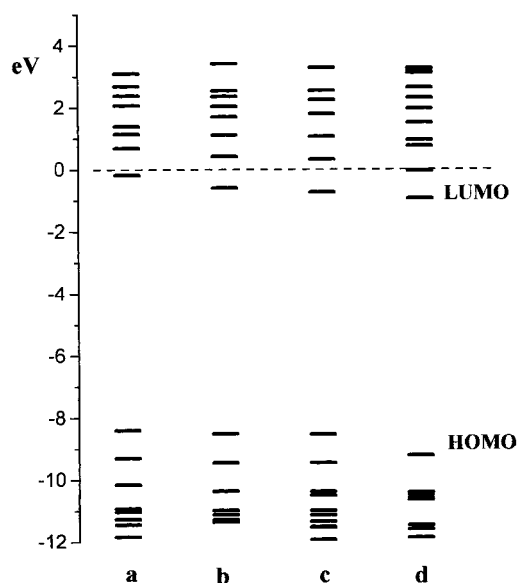

Figure 4. Energy level diagram: (a) retinyl acetate; (b) retinal: (c) methyl retanoate; (d) diketoretinal

Table 2. Calculated heats of formation, C—H bond strengths, radical stabilization energies and propagation rate constants for retinoid autoxidation

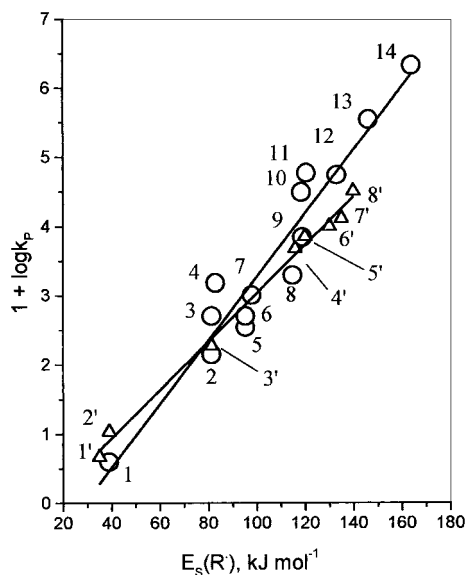| Polyene/bond	ΔH_f (kJ mol^{-1})		$D(\text{R—H})_{calc}$ (kJ mol^{-1}): calculated [Eqn. (1)]	$D(\text{R—H})_{exp}$ (kJ mol^{-1}): experimental (Ref. 11)	$E_S(\text{R}^\cdot)$, (kJ mol^{-1})		k_P, calc. (l mol^{-1} s^{-1})	
	Polyene	Radical			Calculated [Eqn. (2)]	Experimental (Ref. 11)	Abstraction [Eqn. (3)]	Addition [Eqn. (4)]
Retinyl acetate/C15—H	−289.4	−243.5	263.9	275	175.3	164	5.34×10^5	
Retinyl acetate/C4—H	−289.4	−233.3	274.0		165.0		1.8×10^5	
Methyl retinoate/C4—H	−284.3	−229.5	272.8		166.2		2.04×10^5	
13,14-Dihydromethyl retinoate/C13—H	−381.6	−346.4	253.2		185.8			1.03×10^5
Retinal/C4—H	−67.0	−12.0	272.5		166.5		2.1×10^5	
13,14-Dihydroretinal/C13—H	−155.7	−124.3	249.4		189.6			1.4×10^5
2,3-Dihydrodiketoretinal/C2—H	−496.6	−456.1	260.5		178.5			5.75×10^4
Diketoretinal/C14—H	−401.3	−274.6	345.6		93.4		92.9	

Figure 5. Relationships between propagation [for both abstraction (circles) and addition (triangles)] reaction rate constants and stabilization energy of the radicals formed. (1) Cyclohexane; (2) bibenzyl; (3) ethylbenzene; (4) cyclohexene; (5) hept-3-ene; (6) oct-1-ene; (7) diphenylmethane; (8) allyl acetate; (9) penta-1,4-diene; (10) methyl linoleate; (11) methyl linolenate; (12) cyclohexa-1,4-diene; (13) ψ-ionone; (14) retinyl acetate; (1′) but-2-ene; (2′) cyclohexene; (3′) α-methylstyrene; (4′) ethyl sorbate; (5′) β-ionylidene acetalde-hyde; (6′) methyl (all-*E*)-retinoate; (7′) C_{18} ketone; (8′) retinal

the series undergoing hydrogen abstraction by $RO_2^{\cdot}$ during propagation steps are more reactive than polyenes undergoing addition of peroxyls to the polyenic chain.[1] It was concluded that the driving force of the autoxidation process of a polyene is the stabilization energy of the radicals, $E_S(R^{\cdot})$, formed by the attack of $RO_2^{\cdot}$ on the polyene, and this approach was applied to the quantitative description of the autoxidation process. It is essential that the secondary C4—H bonds of any polyene in the carotenoid and retinoid series are not as reactive as the C15—H bonds of retinyl acetate owing to the non-coplanarity of the ring double bond and polyene chain.[6–10] In view of the lack of experimental data, semi-empirical calculations of the heats of formation (ΔH_f) of some retinyl polyenes and polyenyl radicals were performed at the AM1 level and corresponding bond strengths [$D(R—H)$] were calculated from the
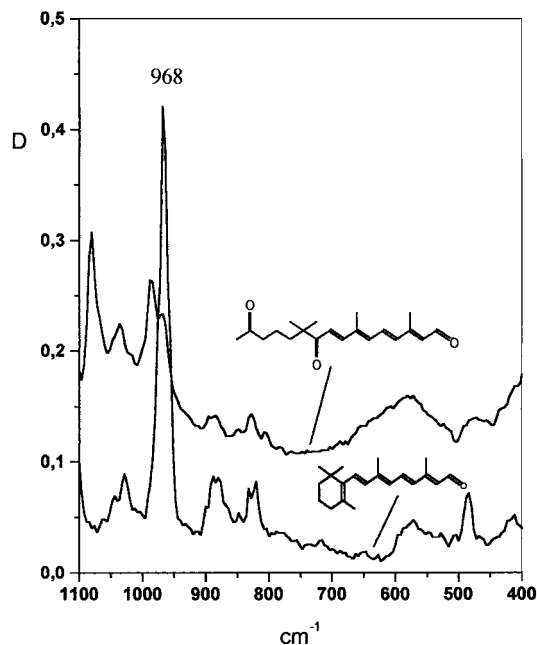

Figure 6. IR spectra of retinal and diketoretinal films

standard equation

$$D(R-H) = \Delta H_f(R^{\cdot}) + \Delta H_f(H) + \Delta H_f(RH) \quad (1)$$

The stabilization energies, $E_S(R^{\cdot})$, of the corresponding radicals were also calculated using the equation

$$E_S(R^{\cdot}) = D(CH_3-H) - D(R-H) \quad (2)$$

The results are summarized in Table 2. The only published experimental value [D(C15—H] for retinyl acetate[11]) is close enough to the calculated value (the difference is about 11 kJ mol^{-1}).

It was shown in our previous studies[1,2] that the reactivity of an organic compound towards peroxy radicals is directly related to the stabilization energy of the radical formed by the abstraction of an allylic hydrogen or by addition of $RO_2^{\cdot}$ to a double bond. Moreover, the data (taken from Ref. 1) presented in Fig. 5 show that at high stabilization energies of radical formation, the rate constants of H atom abstraction are greater than the rate constants of the addition of peroxyls

Table 3. Atom charges of C—H bonds responsible for $\delta_{=C-H}$ bands in IR spectra

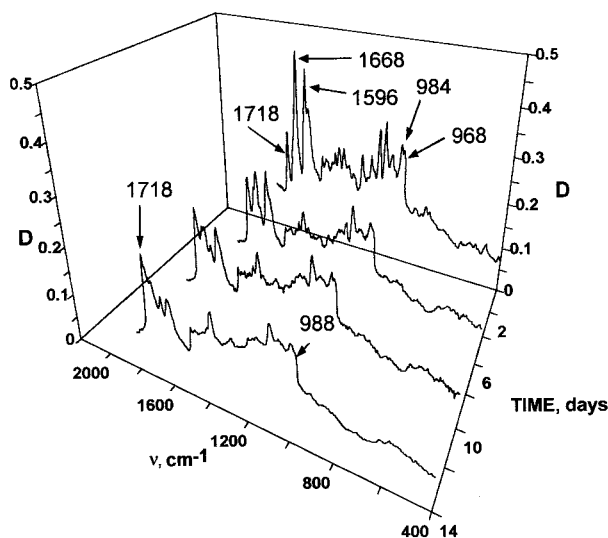	Polyene	Bonds and atom charges						
	C7—H		C8—H		C11—H		C12—H	
Retinal	−0.118	0.127	−0.133	0.129	−0.117	0.128	−0.135	0.130
Retinyl acetate	−0.121	0.127	−0.132	0.127	−0.120	0.130	−0.133	0.1129
Methyl retinoate	−0.118	0.127	−0.133	0.128	−0.144	0.130	−0.137	0.128
	C9—H		C8—H		C5—H		C4—H	
Diketoretinal	−0.218	0.139	−0.076	0.147	−0.117	0.130	−0.129	0.132

Figure 7. Change in the IR spectrum of diketoretinal during autoxidation in air at 25 °C

to the double bonds. The relations are as follows:

$$1 + \log k_P = -1,319 + 0.04586\, E_S(R^\cdot) \quad (3)$$
$$(R = 0.963, SD = 0.417)$$

for propagation by abstraction reactions and

$$1 + \log k_P = -0.452 + 0.0348\, E_S(R^\cdot) \quad (4)$$
$$(R = 0.997, SD = 0.123)$$

for propagation by the addition to double bonds.

The values of propagation rate constants calculated by these equations (Table 2) may be used as a measure of the reactivity in autoxidation of the polyenes under consideration. The values lead to the following sequence of the reactivity decrease: retinyl acetate > retinal > methyl retinoate ≫ diketoretinal. This sequence corresponds to the experimental data on the reactivity of polyenes in solid-state autoxidation (Ref. 2 and this work).

The overall rate of the autoxidation process depends not only on the propagation rate but also on the self-initiation one. It is well known[4] that in the absence of an added initiator, self-initiation of autoxidation occurs due to either the abstraction of a labile H atom from the substrate by O_2 or the disproportionation of two substrate molecules (see also Table 3 in Ref. 2).

The data in Table 1 (Ref. 2) show that thermal initiation rate in solid films of methyl retinoate is only one tenth of that for retinal. It is also seen from Scheme 1 that in diketoretinal as compared with retinal the only labile C—H bond is C14—H, the strength of which is much higher [and $E_S(R^\cdot)$ is much less] than that of C4—H of retinal (Table 2). Consequently, the initiation rate of the autoxidation of **6** is much less than that for retinal. Together with the decrease in propagation rate constant,

this leads to a drastic decrease in the overall rate of autoxidation.

Hence one may conclude that the main reason for the decrease in polyene autoxidation rate in the presence of a carbonyl conjugated to polyene chain is the decrease in the stabilization energy of polyenic radicals formed in the course of the chain process.

It is interesting that a second carbonyl conjugated with the polyene chain (diketoretinal) may also change the IR spectrum of a polyene. All polyenes studied except diketoretinal have a strong band at about 966–968 cm^{-1} corresponding to $\delta_{=C-H}$ vibrations.[1,12,13] The band in the spectrum of diketoretinal is relatively weak and superimposed by other band at 984 cm^{-1} (Fig. 6). As has been shown for retinal,[14] the band is associated with the hydrogen out-of-plane vibrations of HC7=C8H and HC11=C12H. In **6**, the corresponding groups are HC4=C5H and HC8=C9H.

One may suppose that the change in the band on going from retinal (and other poyenes of the series) to **6** is due to the redistribution of atom charges caused by second carbonyl. This is confirmed by AM1 calculations (Table 3). It is also seen (Fig. 7) that during the autoxidation of **6** the polyenic part of the molecule undergoes substantial changes: the bands in the IR spectrum corresponding to the C=C (1596 cm^1) and =C—H (964 and 986 cm^{-1}) bonds and to conjugated carbonyls (1668 cm^{-1}) disappear, whereas the intensity of the unconjugated carbonyl band at 1720 cm^{-1} does not vary. This fact may be regarded as additional evidence for a much higher oxidizability of polyenic compounds as compared with the saturated compounds.

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