

## REACTIVITY OF RETINOIDS AND CAROTENOIDS IN AUTOXIDATION

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The autoxidation of various retinyl polyenes and carotenoids in chlorobenzene at 45°C and in thin solid films on a support at room temperature was investigated. The compounds used were  $\beta$ -carotene, canthaxanthin, retinyl acetate, methyl (all-*E*)-retinoate, methyl (13*Z*)-retinoate, retinal, C<sub>18</sub> ketone,  $\beta$ -ionylidene acetaldehyde,  $\psi$ -ionone,  $\beta$ -ionone and ethyl sorbate as a model compound. It was shown that the isomerization of polyenyl peroxy radicals occurs during autoxidation of all the compounds studied, excepting  $\beta$ -ionone. A kinetic scheme for the polyene autoxidation process was considered and analysed. The conditions under which the rate constants of elementary reactions may be determined were defined and the rate constants of propagation and termination reactions for different polyenes were evaluated. The disappearance and formation of different functional groups were monitored by the spectroscopic investigation of autoxidation of polyene solid films. Mechanisms of the initial stages of the process are proposed for different polyenes.

### INTRODUCTION

Carotenoids and retinoids of different biological activity have been studied intensively for many years. Although oxidation by molecular oxygen (autoxidation) is the main process leading to destruction of these biologically active polyenes during the storage of medicines, its kinetics and mechanism are not yet known in detail. In particular, the rate constants of elementary reactions of various compounds of the series having polyene chains of different lengths have not been determined and compared.

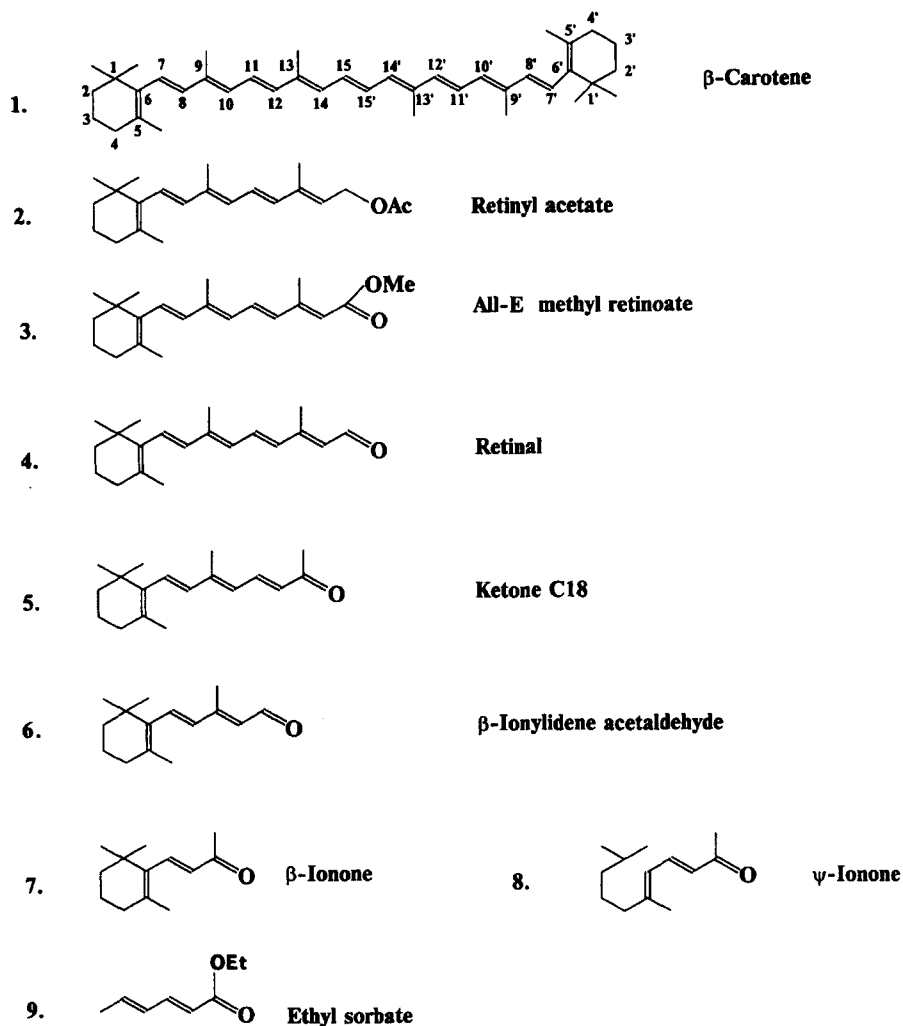
In previous work, some conclusions were drawn about the initial stages of the process<sup>1,2</sup> and various oxidation products formed at fairly high conversion rates of the oxidizing substrates were determined.<sup>2,3</sup> In our previous paper,<sup>4</sup> a brief report of the spectroscopic investigation of some compounds of the series was given. In this paper, we report the results of a study of the kinetics and mechanism of the autoxidation of a series of polyenes bearing polyene chains of different lengths. The main compounds investigated are shown in Scheme 1. For most of the compounds the rate constants of different elementary reactions were determined and the mechanisms of the initial stages of autoxidation of polyenes of various structure were elucidated.

### EXPERIMENTAL

The experimental procedures of polyene purification and storage and for film preparation were described previously.<sup>1,5</sup> Compounds 4–8 (Scheme 1) were obtained by purification of technical products in the usual way. Retinal (4) was separated from its hydroquinone complex by treatment with aqueous NaOH, extracted with diethyl ether and, after evaporation of the ether, recrystallized twice from *n*-hexane. C<sub>18</sub> ketone (5) was purified by column chromatography on silica gel. *n*-Hexane–diethyl ether (9:1) was used for elution. The solvent was evaporated under reduced pressure and residual C<sub>18</sub> ketone dried under vacuum (*ca* 10<sup>-3</sup> mmHg).  $\beta$ -Ionylidene acetaldehyde (6) was purified in a similar manner. The procedure for the purification  $\beta$ -ionone (7) was described in Ref. 6.  $\psi$ -Ionone (8) of 99.7% purity (according to GLC analysis) was obtained by vacuum distillation of the technical product. Ethyl sorbate (9) of analytical grade was used without additional treatment. Methyl (13*Z*)-retinoate was prepared and purified as the all-*E*-isomer<sup>1</sup> using (13*Z*)-retinoic acid.

The rate of oxygen absorption was measured with an automatic recording apparatus operating at constant pressure. The apparatus consisted of a reaction cell, differential contact mercury micromanometer and a hypodermic syringe. All these parts of the apparatus were thermostated by water jackets at 45°C. The connecting capillary tubes were thoroughly insulated by

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Scheme 1

foamed plastic. The piston of the syringe was geared to a synchronous servo motor and a linear potentiometer-recorder system. The imbalance of the pressure was corrected by moving the syringe piston by the servo motor switched on by contact micromanometer through an electronic relay. The displacement of the piston was converted into voltage changes by the potentiometer, and the output signal was recorded by a strip-chart recorder. The sensitivity of the apparatus was about  $10^{-8}$  mol of  $O_2$  per 1 mm of the recorder scale. The electronic spectra of solutions and films were recorded on a Specord M40 instrument (Carl Zeiss, Jena, Germany). The procedures for recording infrared spectra (transmission and multiple attenuated total reflection) were described in Ref. 4.

## RESULTS AND DISCUSSION

### Kinetics of liquid-phase autoxidation

The main peculiarity of the liquid-phase autoxidation of most of the polyenes studied is a non-linear increase in the rate of oxygen absorption rate ( $R_{O_2}$ ) with increase in substrate concentration at a constant initiation rate ( $R_i$ ) (Figure 1). As a result, the relative rate constants evaluated according to the usual kinetic equation

$$R_{O_2} = (k_p/k_t^{1/2})[RH]R_i^{1/2} \quad (1)$$

depend on the substrate concentration  $[RH]$  (Figure 2).

With a view to interpreting the observed dependences, we measured the number of moles of oxygen

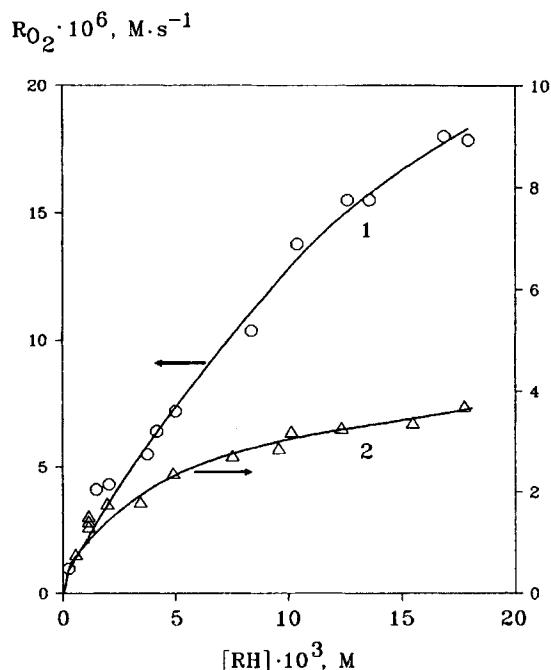


Figure 1. Plot of oxygen absorption rates against polyene concentration for the autoxidation of (1) retinyl acetate and (2) methyl (all-*E*)-retinoate in chlorobenzene at 45 °C and with an initiation rate of  $1 \times 10^{-7} \text{ mol}^{-1} \text{ s}^{-1}$

absorbed per mole of oxidized polyene,  $\Delta n(\text{O}_2)/\Delta n(\text{RH})$ . The results resemble those obtained previously for other polyenes of the series<sup>1</sup> and are presented in Figure 3 and Table 1. It can be seen that the ratio increases when the substrate concentration diminishes. At higher concentrations the ratio decreases and eventually approaches a constant value.

The data obtained indicate that during the oxidation of most polyenes, not only intermolecular but also intramolecular propagation reactions occur.<sup>1</sup> In other words, the polyenyl peroxy radicals may isomerize to give carbon radicals. These carbon radicals react with oxygen, increasing the number of oxygen molecules absorbed per molecule of polyene oxidized. Hence, at low concentrations both propagation reactions occur concurrently, and their ratio depends on the polyene concentration.

The series of elementary reactions of the whole process of initiated autoxidation of polyenes may be described by Scheme 2, where RH represents the polyenic substrate,  $R^{ij\cdot}$  is a carbon radical derived from the substrate and  $R^{ij}\text{OO}\cdot$  is a peroxy radical produced by reaction of  $R\cdot$  with  $\text{O}_2$ ;  $i$  refers to the number of sites attacked and  $j$  to the radical number in a given series. According to the observed dependences, the polyenes

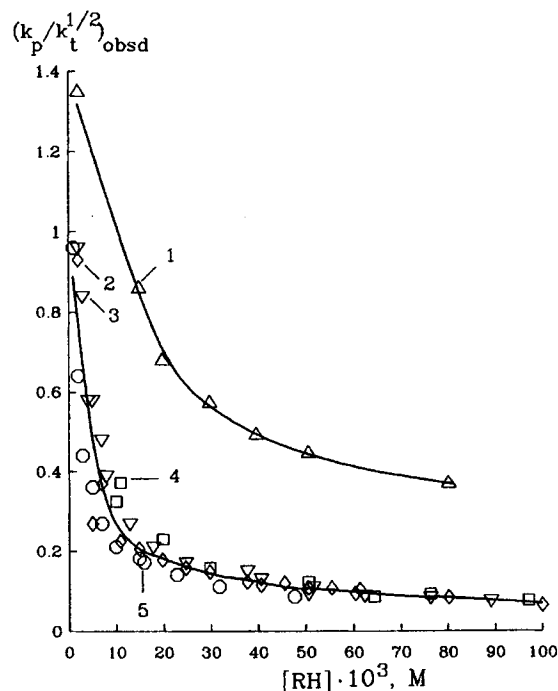


Figure 2. Dependence of the observed rate constant ratios on the concentration of polyenes. Conditions as in Figure 1. (1) Retinyl acetate; (2)  $\text{C}_{18}$  ketone; (3) retinal; (4) methyl (all-*E*)-retinoate; (5)  $\beta$ -ionylidene acetaldehyde

may be divided into different groups (Scheme 3). It is worth noting that only for polyenes with one site attacked by peroxy radicals and one isomerizing radical, i.e. in case when  $i = 1$  and  $j = 1$ , is it possible to find the rate constants of all the elementary reactions.

It is seen that for most polyenes  $i = 1$  and  $j = 1$ . This case has been discussed in detail previously and corresponding kinetic equations were derived.<sup>1</sup> In terms of Schemes 2 and 3 for these compounds, the observed ratio of propagation and termination rate constants evaluated from equation (1) may be

Table 1. Limiting numbers of moles of oxygen absorbed per mole of oxidized polyene

Polyene	$\Delta n(\text{O}_2)/\Delta n(\text{RH})$ extrapolated to [RH] = 0
$\beta$ -Carotene	7
Retinyl acetate	3
Retinal	2
methyl retinoate	2
Methyl (13 <i>Z</i> )-retinoate	2

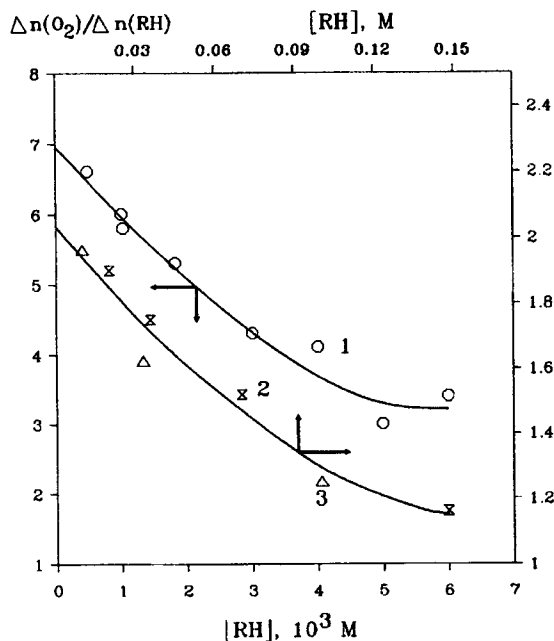
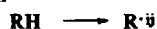


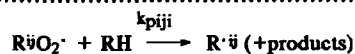
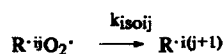
Figure 3. Plot of molar ratios of oxygen absorbed by polyenes during autoxidation versus  $[RH]$ . (1)  $\beta$ -Carotene; (2) methyl (13Z)-retinoate; (3) retinal

#### AUTOXIDATION OF POLYENES

##### Initiation



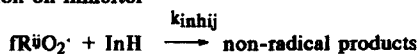
##### Propagation



##### Termination



##### Termination on inhibitor



Scheme 2. Autoxidation of polyenes

expressed as follows:

$$(k_p/k_t^{1/2})_{\text{obsd}} = \frac{k_{p12i}(2k_{iso11} + k_{p11i}[RH])}{(k_{p12i}^2 k_{t1111}[RH]^2 + 2k_{iso11} k_{p12i} k_{t1112}[RH] + k_{iso11}^2 k_{t11212})^{1/2}} \quad (2)$$

where the first two figures of the subscripts to the propagation rate constants denote the radical and the third subscript the site of radical attack on the polyene molecule. In the subscripts to the termination rate constants each pair of figures is referred to one of the reacting radicals. Simplified equations [equations (5), (6) and (8) in Ref. 1] were used to calculate the rate constants of propagation and termination of the autoxidation of compounds 2, 4, 5, 6, 8 and 9. The rate constants for polyene 3 were taken from Ref. 1. The results are presented in Tables 2 and 3.

It can be seen that the rate constants for intermolecular and intramolecular propagation reactions increase

#### KINETIC GROUPS OF POLYENES

$i$  - NUMBER OF SITES ATTACKED

$j$  - NUMBER OF ISOMERIZED RADICALS IN A GIVEN SERIES

$$1. i = 2, j = 0. R_{O_2} = R_{RH}$$

$\beta$ -IONONE

$$2. i = 1, j = 1. R_{O_2} \geq R_{RH}$$

RETINAL  
METHYL RETINOATE  
13-Z METHYL RETINOATE  
KETONE C18  
 $\beta$ -IONYLIDENE ACETALDEHYDE  
 $\Psi$ -IONONE  
ETHYL SORBATE

$$3. i = 1, j = 2. R_{O_2} > R_{RH}$$

RETINYL ACETATE

$$4. i = 1, j = 6. R_{O_2} > R_{RH}$$

$\beta$ -CAROTENE

Scheme 3. Kinetic groups of polyenes

Table 2. Propagation rate constants for autoxidation of polyenes

Polyene	$k_{p_{iji'}} \times 10^{-3} \text{ (1 mol}^{-1} \text{ s}^{-1}\text{)}$			$k_{\text{isoll}} \text{ (s}^{-1}\text{)}$
	$iji' = 131$	$iji' = 111$	$iji' = 121$	
2	$4.2 \pm 0.4$	—	—	—
4	—	$3.2 \pm 1.1$	$4.0 \pm 0.7$	$200 \pm 50$
3	—	$1.3 \pm 0.2$	$2.0 \pm 0.1$	$180 \pm 5$
5	—	$1.0 \pm 0.2$	$2.4 \pm 0.8$	$40 \pm 8$
6	—	$0.7 \pm 0.2$	$1.3 \pm 0.4$	$15 \pm 2$
8	—	$0.7 \pm 0.2$	$0.3 \pm 0.04$	$33 \pm 8$
9	—	$0.5 \pm 0.2$	—	$13 \pm 2$

with polyene chain length. The termination rate constants also increase with increasing unsaturation and reach the diffusion-controlled limit.

### Driving force of the process

The longer the polyene chain, the more stabilized is the polyenyl radical formed by addition of peroxy to the C=C double bond or by abstraction of a labile 'allylic' H atom. Hence it may be concluded that the driving force of the autoxidation process of polyenes is the stabilization energy of polyenyl radicals,  $E_S(R\cdot)$ , formed by attack of  $RO_2\cdot$  on the polyenes under consideration. The values of  $E_S(R\cdot)$  were calculated from the equation<sup>7,8</sup>

$$E_S(R\cdot) = D(\text{CH}_3\text{—H}) - D(\text{R—H}) \quad (3)$$

As shown in Figures 4 and 5, the propagation rate constants for both abstraction and addition reactions of  $RO_2\cdot$  increase linearly with increase in  $E_S(R\cdot)$ . The published data for different organic compounds and those obtained in this work are fitted by the same straight lines. The equations for the lines are as follows: for abstraction reactions:

$$\log k_p = 16.375 - 0.0474D(\text{R—H}) \quad (4)$$

Table 3. Termination rate constants

Polyene	$k_{t_{ijj'}} \times 10^{-8} \text{ (1 mol}^{-1} \text{ s}^{-1}\text{)}$			
	$ijj' = 1313$	$ijj' = 1111$	$ijj' = 1112$	$ijj' = 1212$
2	$0.86 \pm 0.24$	—	—	—
4	—	$56 \pm 34$	$12 \pm 7.0$	$2.6 \pm 1.0$
3	—	$37 \pm 5.0$	$3.0 \pm 1.0$	$0.25 \pm 0.16$
5	—	$6.3 \pm 2.6$	$0.75 \pm 0.27$	$0.09 \pm 0.05$
6	—	$2.3 \pm 0.7$	$0.24 \pm 0.09$	—
	$0.026 \pm 0.005$	—	—	—
8	—	$3.6 \pm 1.7$	$0.71 \pm 0.20$	$0.14 \pm 0.05$
9	—	$1.0 \pm 0.2$	—	—

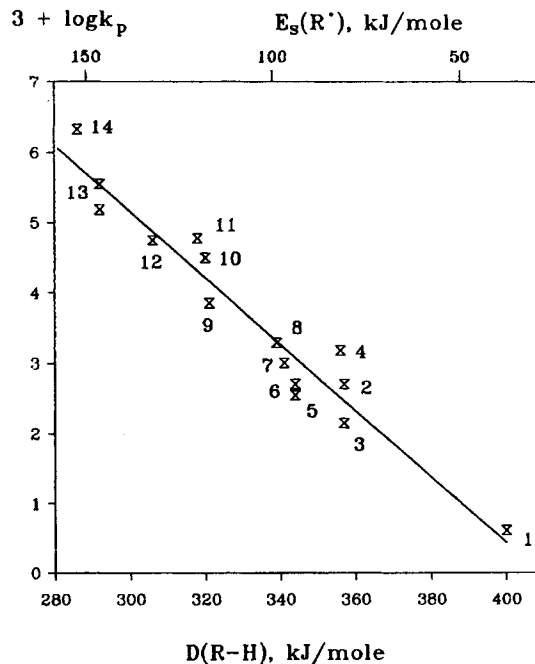


Figure 4. Relationship between propagation reaction rate constants (abstraction reaction) and broken C—H bond energy and stabilization energy of radical formed. (1) Cyclohexane; (2) ethylbenzene; (3) bibenzyl; (4) cyclohexene; (5) hept-3-ene; (6) oct-1-ene; (7) diphenylmethane; (8) allyl acetate; (9) penta-1,4-diene; (10) methyl linoleate; (11) methyl linolenate; (12) cyclohexa-1,3-diene; (13)  $\psi$ -ionone; (14) retinyl acetate. Data for compounds 1–12 from Ref. 9, and for 13 and 14 this work.

for addition reactions:

$$\log k_p = -0.452 + 0.0349E_S(R\cdot) \quad (5)$$

These equations permit the calculation of the rate constants from known  $D(\text{R—H})$  value with reasonable accuracy.

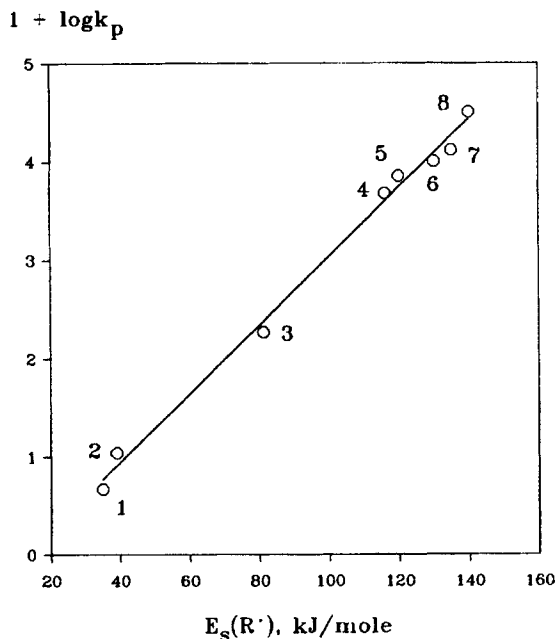


Figure 5. Relationship between propagation rate constants (addition reaction to C=C bond) and stabilization energy of radicals formed. (1) But-2-ene; (2) cyclohexene;<sup>10</sup> (3)  $\alpha$ -methylstyrene;<sup>11</sup> (4) ethyl sorbate; (5)  $\beta$ -ionylidene acetaldehyde; (6) methyl (all-*E*)-retinoate;<sup>1</sup> C<sub>18</sub> ketone; (8) retinal

### Mechanism of polyene autoxidation

To study the mechanism of autoxidation of retinoids and carotenoids, electronic and infrared spectroscopy were used. Autoxidations of solutions, neat liquids and thin solid films on supports were performed. The results obtained were as follows.

During the autoxidation of long polyenes 1, 2, 3 and its 13*Z*-isomer and 4, the main band of polyenic chain in the IR spectrum at 966 cm<sup>-1</sup> ( $\delta_{-C-H}$ ) gradually diminishes and shifts to 970 cm<sup>-1</sup> ( $\delta_{-C-H}$  of type 7 structures) (Figure 6). Simultaneously, the  $\nu_{C-C}$  band in the 1500 cm<sup>-1</sup> region disappears (Figure 7). Bands of carbonyl and hydroxyl groups appear and increase in intensity. The detailed investigation of polyenes revealed that the peroxy radical attack on the retinyl acetate molecule is directed at the H atom at C-15 (abstraction), whereas the carbonyl-containing polyenes [3-6 and (13*Z*)-3] undergo peroxy addition to the last carbon atom of the polyene chain. This conclusion was drawn from the observed formation of non-conjugate carbonyl bands in the IR spectra of oxidizing films of polyenes 3, (13-*Z*)-3 and 4.

The results (Tables 2 and 3) lead to the conclusion that polyenes with 'allylic' hydrogens (2 and 8) are more reactive than polyenes undergoing initial addition of peroxy radicals to the polyene chain (3-6 and 9).

Using the previously derived equation<sup>8</sup>

$$D(R-O_2\cdot) = D(R-H) - 257 \text{ kJ mol}^{-1} \quad (6)$$

and on the basis of structural features of polyenes 1-7 [non-coplanarity of the polyene chain and the ring C=C bond (Figure 8)], it was proposed that sufficiently stable polyenyl peroxy radicals may only be formed by addition of an oxygen molecule to the C-5 carbon atom of corresponding polyenyl radical. This is due to the

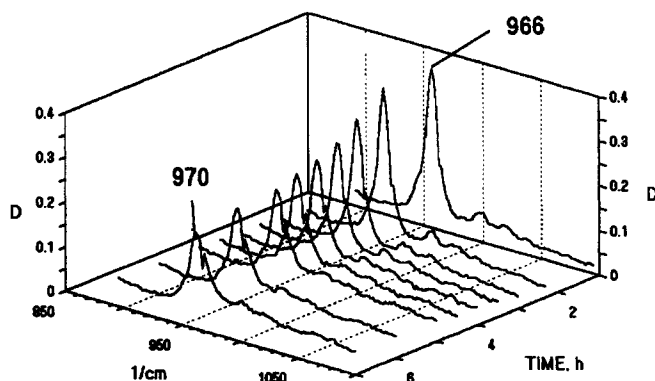


Figure 6. Change of  $\delta_{-C-H}$  band of  $\beta$ carotene during its film autoxidation.

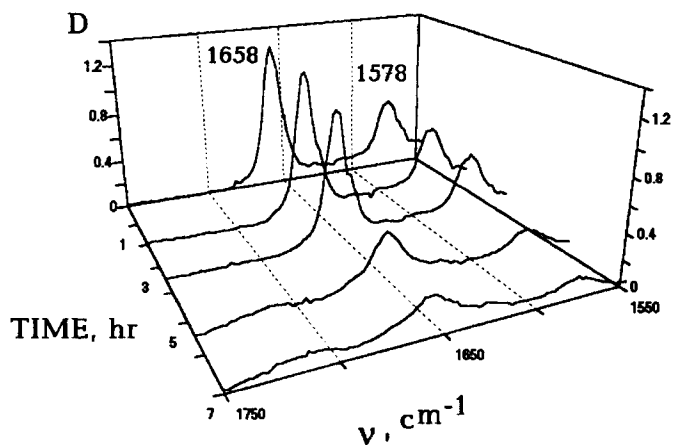
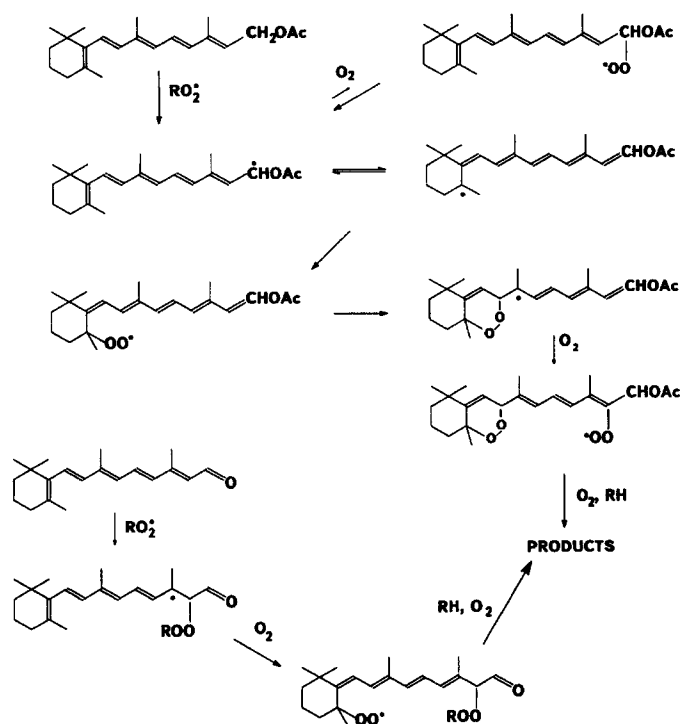


Figure 7. Changes of C=O and C=C bands of retinal during its film autoxidation

PROPOSED INITIAL STAGE MECHANISM OF RETINYL  
ACETATE AND RETINAL AUTOXIDATION



Scheme 4. Proposed initial stage mechanism of retinyl acetate and retinal autoxidation

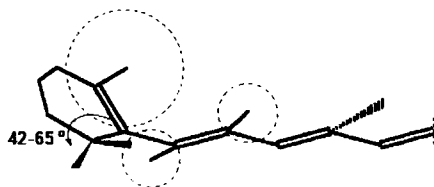


Figure 8. Steric effects and conformation of retinoid and carotenoid molecules<sup>12-15</sup>

reversibility of the polyenyl peroxy radical formation reaction:



In the case of polyenyl radicals formed from compounds 1-5, the equilibrium is shifted to the left even at room temperature. The energetically favourable reaction course is addition of an oxygen molecule to the C-5 atom of the retinoid or carotenoid polyenyl radical. Owing to the non-coplanarity of the ring double bond and polyene chain (Figure 8), the energy gain by C-5—O<sub>2</sub>· rupture is much smaller than those for C-15—O<sub>2</sub>· or any other C—O bond of the peroxy radicals under consideration and the C-5—O<sub>2</sub>· peroxy radical is the most stable of all the peroxy radicals initially formed from a given polyene.

The proposed mechanisms for the initial stages of polyene autoxidation are shown in Scheme 4.

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