

# CYCLIZATION REACTIONS OF CAROTENOIDS

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## ABSTRACT

Acetonolysis of the titanium tetrachloride complex of lycopene gives  $\delta$ -carotene and  $\epsilon$ -carotene. Analogous cyclization reactions have been achieved with  $\gamma$ -carotene, rubixanthin esters and  $\beta$ -zeacarotene.

In some typical parasitic plants and semi-parasitic plants, carotenoids with  $\alpha$ -structures predominate over those with  $\beta$ -structures.

The formation of carotenoid epoxides is favoured in plants grown at high altitudes.

$\beta$ -Carotene inhibits the autoxidation of aldehydes. The products include a new type of carotenoid epoxide in which the epoxide group is situated along the polyene chain. Peracid oxidation of canthaxanthin yields the 13,14-epoxide.

Treatment with lead tetra-acetate constitutes a convenient method for the conversion of carotenoids into their furanoid oxides.

## INTRODUCTION

In recent years the attention of many organic and biochemists has been directed towards the attempt to contribute by *in vitro* synthesis to the elucidation of certain steps of presumed or proposed biosynthetic pathways. Such attempts have been undertaken also in the field of carotenoids and I should like to report here on *in vivo* and mainly *in vitro* cyclization reactions of natural carotenoids leading to cyclic carotenoids with  $\alpha$ - and  $\beta$ -ionone rings, epoxides and carotenoids with furan rings.

## CAROTENOIDS WITH $\alpha$ AND $\beta$ IONONE RINGS

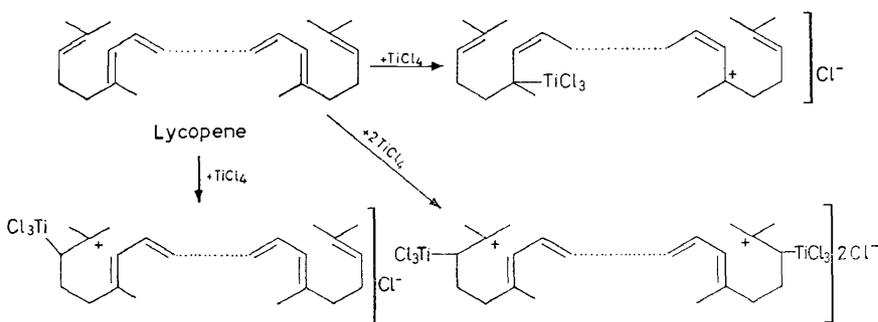
Considering firstly the cyclization reactions leading *in vivo* to carotenoids with  $\alpha$ - and  $\beta$ -ionone rings, there seems little doubt that straight chain hydrocarbon carotenoids undergo cyclization reactions. Supporting evidence obtained in experiments using cell-free preparations or extracts of microorganisms, was reviewed only two years ago by Porter and Anderson<sup>1</sup>. As for the acyclic hydrocarbon carotenoids presumed to be the direct precursors of cyclic carotenoids, I would only briefly refer to the conversions proposed especially by Porter and co-workers<sup>2, 3</sup>, and by Goodwin<sup>4</sup>, shown in *Figure 1*. A general reaction mechanism for the formation of  $\alpha$ - and  $\beta$ -carotenes proposed by Goodwin<sup>5</sup> is shown in *Figure 2*. But there is no direct evidence in favour of such cyclization reactions. So the question arose whether acyclic carotenoids can be converted *in vitro* into cyclic carotenoids with  $\alpha$ - and  $\beta$ -ionone rings.



## CYCLIZATION REACTIONS OF CAROTENOIDS

Attempts in this direction were encouraged by successful cyclizations of compounds related to carotenoids. For example, as it is shown in *Figure 3*,  $\psi$ -ionone, with the terminal system like that of lycopene and other acyclic carotenoids, undergoes cyclization under the action of  $H_2SO_4$  or  $H_3PO_4$ <sup>6</sup>. A mixture consisting mainly of  $\alpha$ - and  $\beta$ -ionone is formed in which  $\beta$ -ionone is formed predominantly from  $\alpha$ -ionone. Primarily the ratio  $\alpha$ : $\beta$ -ionone is 9:1,  $\alpha$ -ionone being formed seven times as fast as  $\beta$ -ionone. The isomerization of  $\alpha$ - to  $\beta$ -ionone is irreversible.

We failed to observe a cyclization from the action of  $H_2SO_4$  and  $H_3PO_4$  upon the easily available lycopene. But investigating the action of another agent upon lycopene, namely the action of  $TiCl_4$  which is known to exhibit a pronounced tendency to form charge transfer complexes, we have established that there is formation of a mixture of complexes<sup>7</sup>. In these complexes  $TiCl_4$  is fixed either to the 5,6 double bond or to an isolated double bond, 1,2 and 1',2' (*Figure 4*). The complexes are easily split by acetoneolysis yielding in the case of the complex 1, dehydrolycopene; in the case of complex 2,  $\delta$ -carotene; and in that of complex 3,  $\epsilon$ -carotene. The latter is also formed by the action of  $TiCl_4$  on  $\delta$ -carotene, followed by acetoneolysis (*Figure 5*). The mechanisms (*Figure 6*) of these cyclizations are, of course, similar to those shown in *Figures 3* and *4*.



*Figure 4.* Formation of  $TiCl_4$  lycopene complexes. (V. Tămaş and C. Bodca, 1968.)

We have tried also the cyclization of carotenoids other than lycopene and  $\delta$ -carotene. With  $TiCl_4$  acting on  $\gamma$ -carotene, we obtained  $\alpha$ -carotene and  $TiCl_3$ -catalyzed cyclization of rubixanthin-ester resulted in 3-hydroxy- $\alpha$ -carotene after saponification (*Figure 7*). We have isolated  $\alpha$ -zeacarotene from corn and tried to cyclize this presumed precursor of the cyclic carotenoids. Among the reaction products obtained by the acetoneolysis of the  $TiCl_4$ -complex, we isolated  $\alpha$ -carotene and 7',8'-dihydro- $\alpha$ -carotene (*Figure 8*). So we can say today that acyclic carotenoids can be converted *in vitro* into cyclic carotenoids and we regard this as support of the *in vivo* cyclization schemes shown in the first slides (*Figures 3, 4* and *5*).

We have seen that in all the cyclizations by  $TiCl_4$  so far achieved  $\alpha$ -ionone rings are always formed. If we consider also the results obtained in the cyclization reactions of  $\psi$ -ionone it can be concluded that the  $\alpha$ -ionone ring

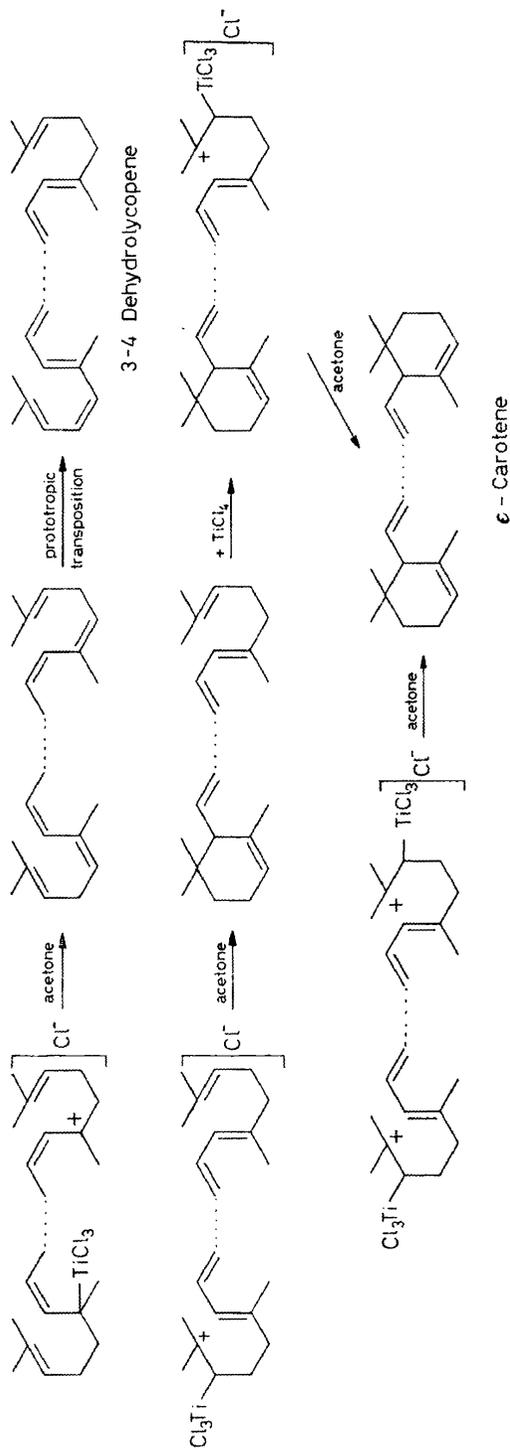


Figure 5. Conversion of  $TiCl_4$  lycopene complexes. (V. Támas and C. Bodea, 1968.)

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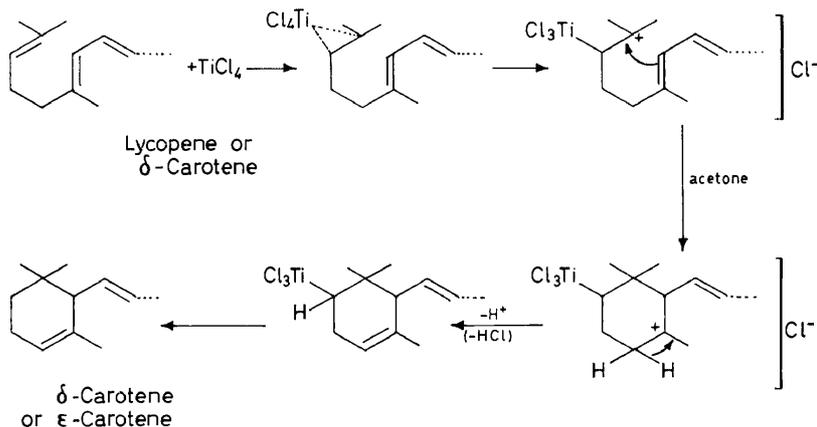


Figure 6. Cyclization mechanism under the action of  $\text{TiCl}_4$ . (V. Tămaş and C. Bodea, 1968.)

is formed more easily than the  $\beta$ -ionone ring, at least by *in vitro* cyclizations. But we believe that some indications in this regard can be found *in vivo* also. We have recently examined the carotenoids of some typical parasitic and semi-parasitic plants with a lower metabolism as compared to that of autotrophic plants<sup>8</sup>. The number of carotenoids with the  $\alpha$ -structure is much higher than that of carotenoids with the  $\beta$ -structure (Tables 1 and 2). At any rate we have the feeling that it may be of interest to continue the studies on *in vitro* cyclization of natural carotenoids. We hope to be able to collect this summer sufficient quantities of *Cantharellus tubiformis*, to get by extraction the necessary quantities of neurosporene to try the cyclization of this presumed precursor of cyclic carotenoids with  $\alpha$ - and  $\beta$ -ionone rings.

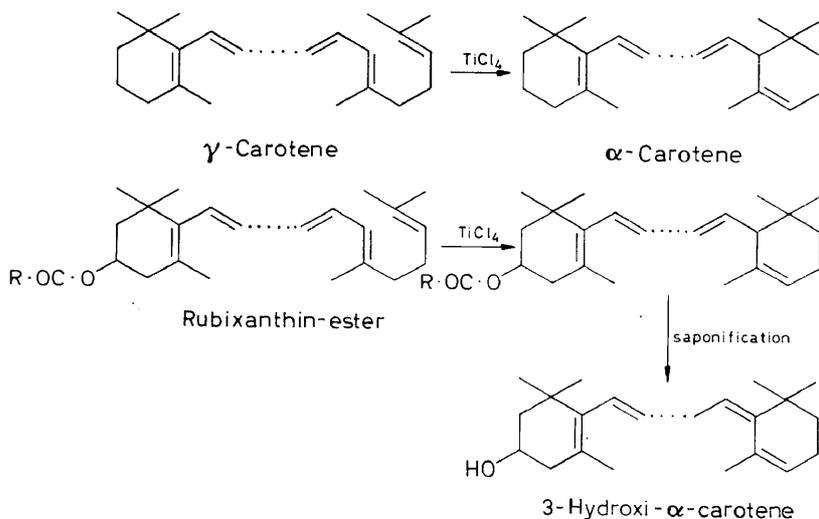


Figure 7. Cyclization of  $\gamma$ -carotene and rubixanthin-ester. (V. Tămaş, G. Neamtu and C. Bodea, 1969.)

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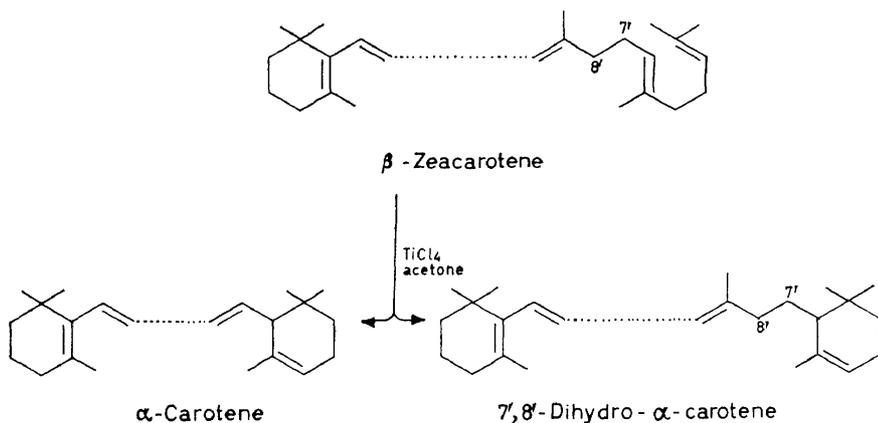


Figure 8. Cyclization of  $\beta$ -zeacarotene. (V. Tămaş and C. Bodea, 1969.)

Table 1. Carotenoids in the flowers of *Orobanche* species (parasites) (mg/100 g)

Carotenoid	<i>Orobanche</i>				
	<i>lutea</i>	<i>purpurea</i>	<i>gracilis</i>	<i>vulgaris</i>	<i>alba</i>
<i>with <math>\alpha</math>-Structure</i>					
$\alpha$ -Carotene	0.02	—	trace	trace	—
Lutein, ester	0.03	0.30	0.28	0.42	0.23
Eloxanthin, ester	0.70	0.55	0.35	0.60	0.40
Flavoxanthin, ester	0.40	0.45	0.30	0.43	0.35
<i>total</i>	1.15	1.30	0.93	1.45	0.98
<i>with <math>\beta</math>-Structure</i>					
$\beta$ -Carotene	0.08	0.20	0.14	0.25	0.12
Violaxanthin, ester	0.25	—	0.18	0.30	—
<i>total</i>	0.33	0.20	0.32	0.55	0.12

Table 2. Carotenoids in the flowers of *Rhinanthus* species and *Melampyrum* species (semiparasites) (mg/100 g)

Carotenoid	<i>Rhinanthus</i>		<i>Melampyrum</i>	
	<i>major</i>	<i>minor</i>	<i>silvaticum</i>	<i>bihariense</i>
<i>with <math>\alpha</math>-Structure</i>				
Lutein	1.20	1.40	0.80	0.50
Lutein, ester	0.20	0.40	1.58	1.00
Eloxanthin	0.35	—	0.40	0.32
<i>total</i>	1.75	1.80	2.78	1.82
<i>with <math>\beta</math>-Structure</i>				
$\beta$ -Carotene	0.80	0.56	0.50	0.28

## CAROTENOID EPOXIDES

Let us now turn to the carotenoid epoxides. It is known that epoxides of carotenoids are not only widely distributed in nature, but they undoubtedly play important roles, being involved it seems in electron transport, in protection against lethal photosensitized oxidations, in conversions of carotenoids, and maybe in the formation of xanthophylls. Probably most of these reactions are enzymatical ones, as it was pointed out by Krinsky *et al.*<sup>9</sup> Little is yet known about the *in vivo* formation of carotenoid epoxides<sup>1</sup>. There are probably more epoxides in various plants than we know today and it looks as if light and especially ultra-violet light would favour the formation of epoxides *in vivo*. We have some indications of this from examination of a large number of plants which usually grow at an altitude of 1500 to 2000 meters<sup>10</sup> (Table 3).

The oxygen concentration in these regions is lower, but there is more ultraviolet light and as it is to be seen, the content of epoxides and xanthophylls is much higher than that of hydrocarbon carotenoids. Better evidence concerning the influence of the altitude upon the content of epoxides has been obtained by examining the same plant grown at different elevations.<sup>11</sup> The carotenoids of the flowers of *Caltha laeta* and of *Taraxacum officinale* collected at different elevations are shown in Figures 9 and 10.

How does one explain epoxidation of carotenoids *in vivo* from *in vitro* experiments? Considering that in plant tissues carotenoids occur along with many compounds which may undergo autoxidation and that carotenoids can act as antioxidants, we thought that it may be of interest to take a

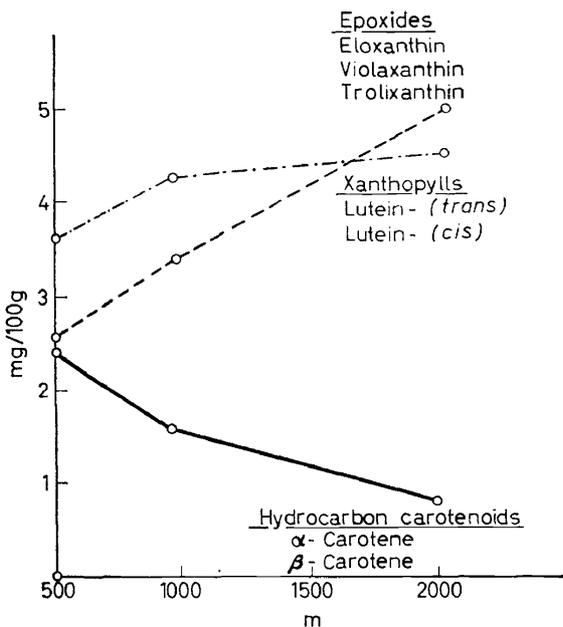


Figure 9. *Caltha laeta* grown at 500, 900 and 1800–2000 m. Flower carotenoids (mg/100 g). (G. Neamțu and C. Bodea, 1965.)

Table 3. Plants grown at a height of 1500–2000 m flower carotenoids (mg/100 g)

Carotenoid	Plants					
	<i>Genista oligosperma</i>	<i>Ranunculus flabellifolius</i>	<i>Hieracium flarzsky</i>	<i>Potentilla ternata</i>	<i>Genum montanum</i>	<i>Hipchoeris uniflora</i>
<i>Hydrocarbons</i>						
α-Carotene	0.08	—	0.028	0.06	0.04	—
β-Carotene	1.20	0.76	0.26	1.20	1.20	0.70
<i>Total</i>	1.28	0.76	0.288	1.26	1.24	0.70
<i>Xanthophylls</i>						
Lutein	2.70	1.43	1.02	0.63	0.76	0.80
Cryptoxanthin	—	—	0.18	0.20	0.40	—
Zeaxanthin	—	—	2.50	1.00	0.85	0.30
<i>Total</i>	2.70	1.43	3.70	1.83	2.01	1.10
<i>Epoxides</i>						
Eloxanthin	0.60	0.86	2.56	2.00	1.50	1.20
Cryptoxanthin-epoxide	—	—	—	0.40	—	—
Antheraxanthin	—	0.12	0.70	0.26	0.12	—
Violaxanthin	1.40	0.26	1.58	0.89	0.34	0.40
Flavoxanthin	0.80	0.14	1.30	1.40	—	0.50
<i>Total</i>	2.80	1.38	6.14	4.95	1.96	2.10

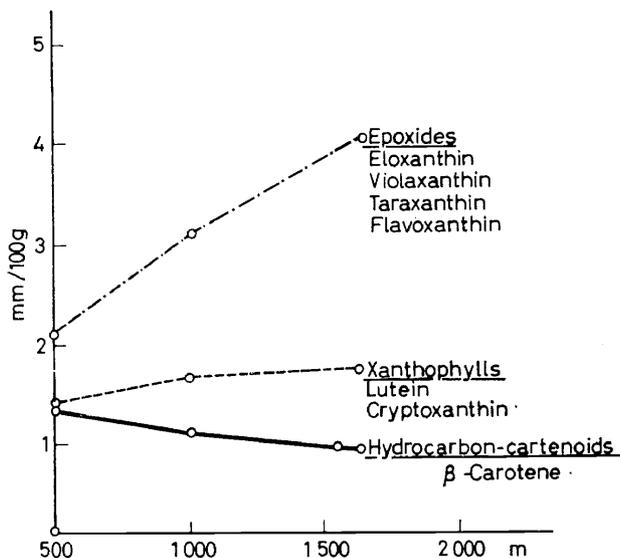


Figure 10. *Taraxacum officinale* grown at 500, 1000 and 1500–1600 m. Flower carotenoids (mg/100 g). (G. Neamtu and C. Bodea, 1965.)

closer look at the mechanism of the intervention of carotenoids in autoxidation reactions. We have chosen as a model the autoxidation of aldehydes and we followed the autoxidation reaction by measuring oxygen absorption. We have worked with acetaldehyde, benzaldehyde, and butyraldehyde, and the results in all cases are nearly the same.

We have shown in Figure 11 autoxidation of a solution of acetaldehyde in benzene. When no other substance is present the oxygen absorption starts almost immediately. When small amounts of  $\beta$ -carotene or another carotenoid are added the oxygen absorption is almost completely blocked. During

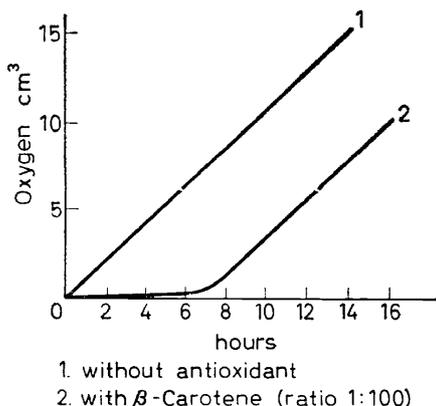
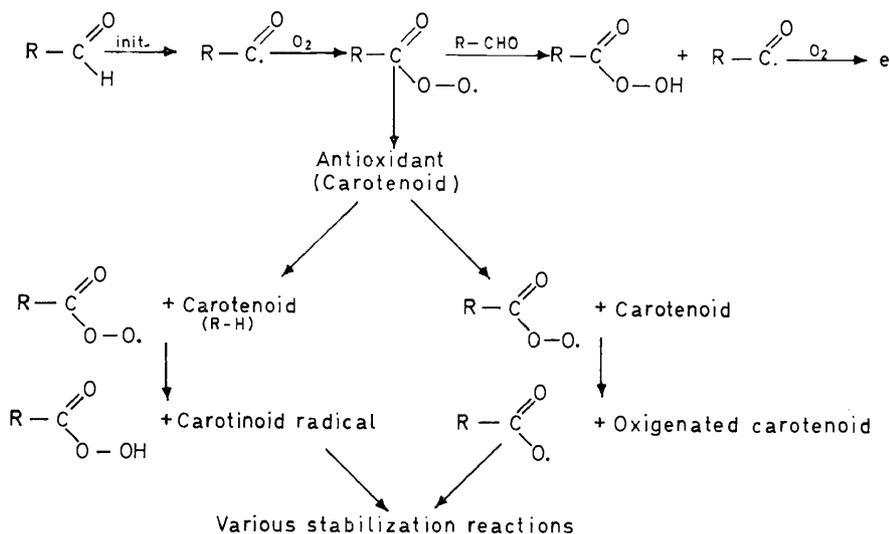


Figure 11. Autoxidation of acetic aldehyde in benzene. (E. Nicoară and C. Bodea, 1968.)

this period a continuous decolouration of the solution takes place until the solution becomes colourless and then the oxygen absorption starts.

Let us now consider the mechanism of inhibition of autoxidation by carotenoids. It is well known that the autoxidation of aldehydes is a chain reaction which follows the steps shown in the *Figure 12*, the main promoters



*Figure 12.* Inhibition of the autoxidation of aldehydes. (E. Nicoară and C. Bodea, 1968.)

of the reaction being the peroxy radicals. In the presence of a carotenoid as antioxidant an interaction takes place between the peroxy radicals and the carotenoid. Two different mode of actions must be considered. One consisting of the removal by the peroxy radical of an active hydrogen atom from the allylic methylene of the carotenoid, leading to a carotenoid radical and a peracid, and the other mechanism consisting of the transfer of the peroxygen atom of the peroxy radical to a double bond of the carotenoid with formation

of an oxygenated carotenoid and the radical  $\text{R}-\text{C} \begin{array}{l} \text{O} \\ \parallel \\ \cdot \\ \text{O} \end{array}$  This radical as

well as the carotenoid radical, undergoes various stabilization reactions.

Analyzing the reaction products in the case of  $\beta$ -carotene acting as antioxidant, we obtained the reaction scheme shown in *Figure 13*. If the first mechanism shown in *Figure 12* is operative, removal of an H from position 4 and 4' of  $\beta$ -carotene will form a mono- and diradical of  $\beta$ -carotene. By coupling with a hydroxyl radical the monoradical is converted into isocryptoxanthin. The hydroxyl radical can have its origin in the splitting of the peracid formed in the autoxidation reaction<sup>12</sup> or in traces of water. The diradical of  $\beta$ -carotene is stabilized by conversion into retrodehydrocarotene.

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Through the second inhibition mechanism, consisting of the interaction of the per-oxygen atom with the double bonds of the carotenoid, there is formed a 5,6-epoxide, which undergoes isomerization due to the acid medium resulting in the formation of 5,8-epoxide. This means that carotenoids also can be converted into 5,6-epoxides *in vivo* by autoxidation processes taking

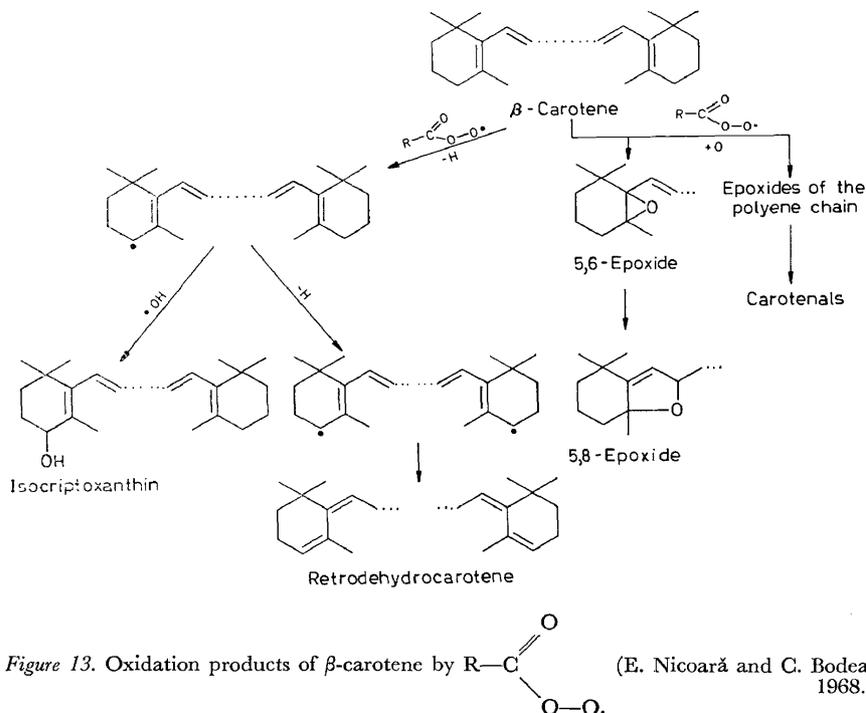


Figure 13. Oxidation products of  $\beta$ -carotene by  $\text{R}-\text{C}(=\text{O})-\text{O}-\text{O}\cdot$  (E. Nicoară and C. Bodea, 1968.)

place in the presence of carotenoids. Besides these known epoxides we found other epoxides with different properties. We obtained for the first time indications of the existence of epoxides on the polyene chain. These epoxides are probably the precursors of degradation products like the various carotenals found among the reaction products. The findings summarised in Figure 13 show that many reaction products are formed during the autoxidation of aldehydes in the presence of  $\beta$ -carotene. Therefore it was not possible to obtain large quantities of the epoxides of the polyene chain, which were of particular interest to us. So we decided to choose as antioxidant another carotenoid having a carbonyl group in the allylic position on the  $\beta$ -ionone ring. Such a compound seems to offer two advantages as compared to  $\beta$ -carotene. No hydrogen removal could take place and no formation of 5,6-epoxides was expected, as it is known from the investigations of Bodendorf<sup>13</sup> that a carbonyl group renders the epoxidation of the conjugated double bond more difficult. These were the reasons leading us to use canthaxanthin as an antioxidant (Figure 14).

As it was expected in the experiments with canthaxanthin as antioxidant, fewer reaction products were formed than in the experiments with  $\beta$ -

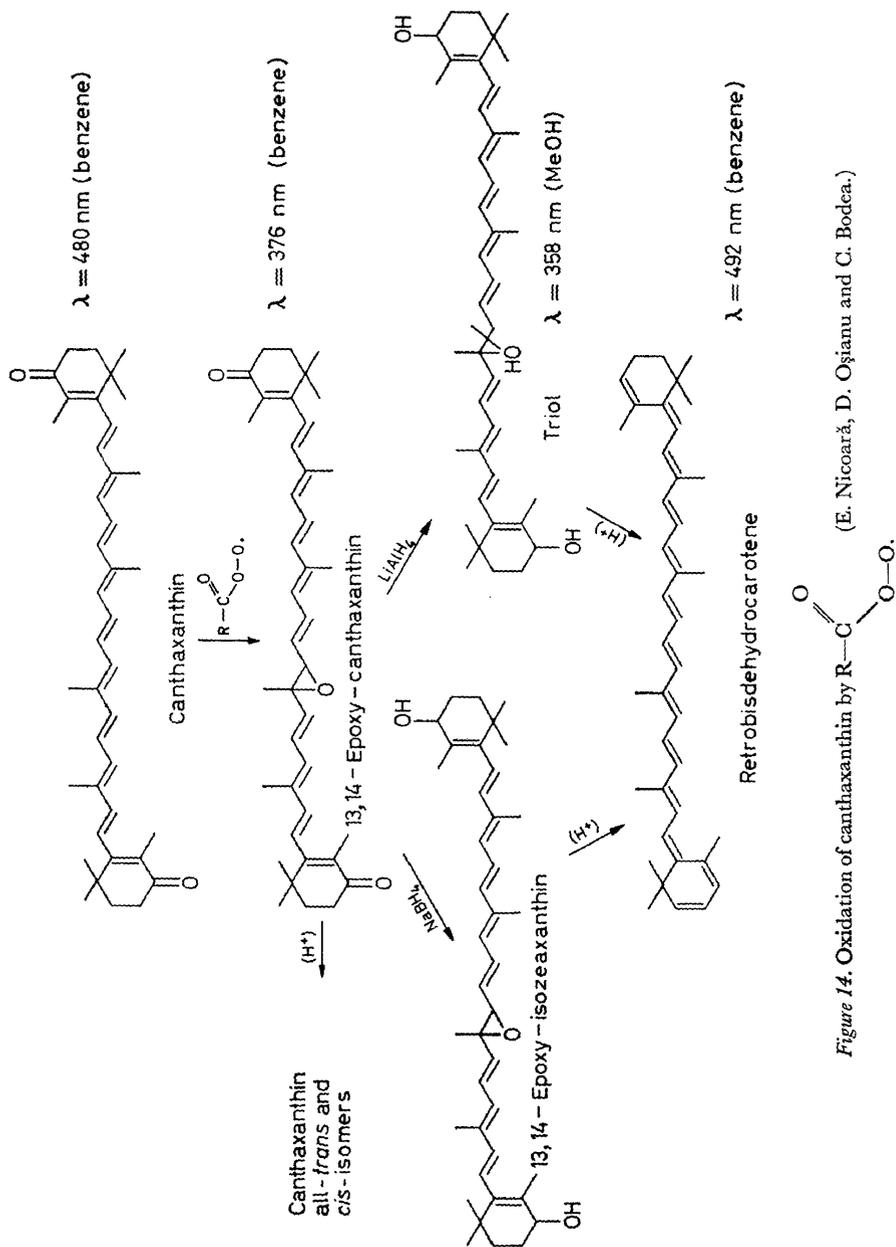


Figure 14. Oxidation of canthaxanthin by  $\text{R}-\text{C}(=\text{O})-\text{O}-\text{O}-$  (E. Nicoară, D. Oşianu and C. Bodea.)

## CYCLIZATION REACTIONS OF CAROTENOIDS

carotene but larger quantities of epoxides of the polyene chain were found. By chromatographic separation we successfully isolated a monoepoxide of the polyene chain which is not converted by acids into a 5,8-epoxide like the hitherto known 5,6-epoxides, but regenerates easily the original canthaxanthin along with a *cis* isomer of canthaxanthin. To obtain information about the behaviour and structure of this monoepoxide we reduced it with  $\text{NaBH}_4$  which is known not to act upon the epoxide group. The isozeaxanthin epoxide thus formed showed the same behaviour as the canthaxanthin epoxide, no 5,8-epoxide being formed in acid medium. As expected the allylic OH groups were eliminated by dehydration, along with the loss of the epoxidic oxygen, thus resulting in retrobisdehydrocarotene. The presence of a single epoxide ring was deduced from the determinations of relative polarity of the canthaxanthin epoxide and of the reduction product with  $\text{LiAlH}_4$ , according to the Krinsky method<sup>14</sup>.  $\text{LiAlH}_4$  is known to reduce the carbonyl group as well as the epoxide into OH groups. The reduction product showed a relative polarity corresponding to three hydroxyl groups. From the absorption spectra of the epoxide and of the reduction product it could be concluded that the double bond which suffered epoxidation is the 13,14-double bond.

We obtained the 13,14-epoxide, along with other epoxides of the polyene chain, in higher yields when canthaxanthin was reacted with perbenzoic acid. Studies on the localization and the properties of these epoxides are now in progress and we hope to be able to communicate these data soon. In our opinion there are no reasons why epoxides of the polyene chain could not exist *in vivo* too. That they have not yet been identified in nature may be explained by the ease with which they lose the epoxidic oxygen. Their biological role may lie in this ease with which they capture and release the active oxygen.

## CAROTENOIDS WITH FURAN RINGS

Finally some comments on carotenoids with furan rings or 5,8-epoxides. As mentioned 5,6-epoxides are converted *in vitro* and maybe also *in vivo* by acids into 5,8-epoxides. The yields with which 5,6-epoxides can be prepared *in vitro* by the action of peracids are small, and consequently the yields of 5,8-epoxides are even smaller. We obtained 5,8-epoxides in much better yield by the action of  $(\text{CH}_3\text{COO})_4\text{Pb}$  on some carotenoids. For example if  $\beta$ -carotene is treated with  $(\text{CH}_3\text{COO})_4\text{Pb}$ , citroxanthin is formed along with other reaction products<sup>15</sup> (Figure 15). According to the reaction mechanism of  $(\text{CH}_3\text{COO})_4\text{Pb}$  upon a double bond given by Criegee<sup>16</sup>, the following mechanism seems probable: fixation of an acetyl ion on the 5,6 double bond; formation of a 5,6-epoxide which cannot be isolated; and conversion of this into the 5,8-epoxide. By the same method we have prepared 5,8-epoxides from cryptoxanthin, zeaxanthin and other carotenoids. It is now well established that *in vivo* the 5,6-epoxides can lose the oxygen atom to regenerate by de-epoxidation the original carotenoid, as for example in the well known reversible system zeaxanthin-antheraxanthin-violaxanthin. We have found that certain 5,8-epoxides can undergo de-epoxidation also.

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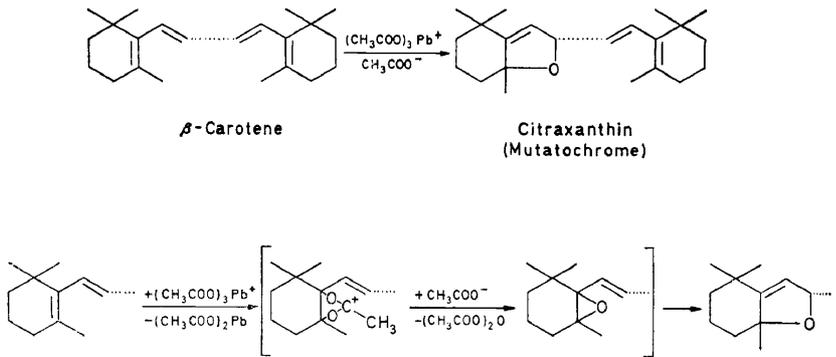


Figure 15. Action of  $(\text{CH}_3\text{COO})_4\text{Pb}$  on  $\beta$ -carotene. (C. Bodea and E. Nicoară, 1962.)

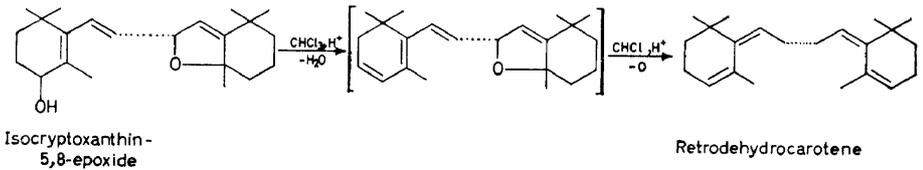


Figure 16. De-epoxidation of isocryptoxanthin-5,8-epoxide. (C. Bodea and E. Nicoară, 1962.)

For instance the 5',8'-isocryptoxanthin-epoxide is dehydrated in acid chloroform into a dehydro product which undergoes spontaneous de-epoxidation to give retrodehydrocarotene (Figure 16). Whether de-epoxidations of 5,8-epoxides can occur *in vivo* also, is a question we cannot answer for the time being.

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