Carotenoid 5,6-, 5,8- and 3,6-epoxides

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Dedicated to Professor Sándor Antus on the occasion of his 60th birthday

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Abstract

The isolation, semisynthesis and structure elucidation of carotenoid 5,6-, 5,8- and 3,6-epoxides as well as their derivatives are discussed in detail.

This review gives a brief summary of the plausible biosynthetic transformations of the 5,6-epoxy-β-ring. A wide variety of carotenoid end-groups can be obtained from 5,6-epoxy carotenoids. In order to get information on the enzyme activities and regulation of the biosynthetic pathway of carotenoids some of the intermediate steps of these transformations were investigated (e.g. formation of allenic, κ- and γ-end-groups as well as ring opening reactions). The ring opening of the 3-hydroxy-5,6-epoxy end group, resulting in the formation of 3,5,6-trihydroxy compounds, was studied in detail in order to obtain evidence for the proposed reaction mechanisms of the two different biosynthetic routes (acid or enzyme catalysis) which also explain the configurations at the C(5) and C(6) atoms.

Keywords: Carotenoid, 5,6-epoxides, 3,6-epoxides, isolation, structure elucidation

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Contents

Introduction

Introduction

Carotenoids are among the most common natural pigments, and more than 600 different compounds have been characterized. Carotenoids are responsible for many of the red, orange, and yellow colors of plant leaves, flowers and fruits, as well as the colors of some birds, insects, fish and crustaceans. Only plants, bacteria, fungi, and algae can synthesize carotenoids, but many animals incorporate them from their diet.[1]

The biological properties of carotenoids can be divided into functions, which are essential to the well-being of organisms, and actions or associations, which are either responses to the administration of carotenoids, or are phenomena which still lack evidence of causal relationship to the presence of carotenoids.[2] Carotenoids have two major functions in photosynthesis: they serve as accessory pigments for light harvesting and in the prevention of photooxidative damage. Carotenoids can also have antioxidant properties (e.g. in animals), and the so-called provitamin A carotenoids are used as sources of vitamin A.

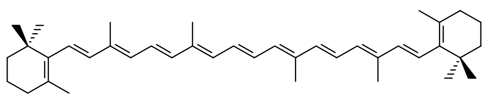
The unique structure of carotenoids determines their potential biological functions and actions. Most carotenoids can be derived from a 40 carbon basal structure including a system of conjugated double bonds. The central chain may carry cyclic end-groups that can be substituted with oxygen-containing functional groups. Based on their composition, carotenoids are divided into two classes, carotenes containing only carbon and hydrogen atoms, and oxocarotenoids (xanthophylls) carrying one or more oxygen atoms. Hydroxy groups are common, particularly in position 3 or 4. Many carotenoids possess an epoxy group, usually in positions 5,6 or 5,8 (the 5,8-epoxides are often referred to as furanoids or furanoid oxides), though some 1,2-epoxides, 3,6-epoxides and 4,5-epoxides have been reported also. Carotenoids containing aldehyde, keto, carboxy, carbomethoxy, and methoxy groups, acetates and lactones are also encountered.[3]

This review gives a summary concerning the carotenoid 5,6-, 5,8- and 3,6-epoxides as well as the isolation, semisynthesis and structure elucidation of the above mentioned carotenoids and their derivatives, paying special attention to the results obtained in our laboratories.

1. 5,6-Epoxy-carotenoids

A major class of pigments in higher plants comprises the 5,6-epoxides of β-carotene (**1**) and zeaxanthin (**2**) and related carotenoids in which one of the end groups is identical with either a β-ring or a 3-hydroxy- β-ring.[4] All green leaves contain violaxanthin ((3*S*,5*R*,6*S*,3′*S*,5′*R*,6′*S*)-5,6,5′,6′-diepoxy-5,6,5′,6′-tetrahydro-β,β-carotene-3,3′-diol, **3**) and neoxanthin ((3*S*,5*R*,6*R*, 3′*S*,5′*R*,6′*S*)-5′,6′-epoxy-6,7-didehydro-5,6,5′,6′-tetrahydro-β,β-carotene-3,5,3′-triol, **4**).[5]

Antheraxanthin ((3*S*,5*R*,6*S*,3′*R*)-5,6-epoxy-5,6-dihydro-β,β-carotene-3,3′-diol, **5**), lutein 5,6-epoxide ((3*S*,5*R*,6*S*,3′*R*,6′*R*)-5,6-epoxy-5,6-dihydro-β,β-carotene-3,3′-diol, **6**)[6], and a variety of other 5,6-epoxy carotenoids (β-carotene 5,6-epoxide (**7**), β-carotene 5,6,5′,6′-diepoxide (**8**), β-cryptoxanthin 5,6-epoxide (**9**), β-cryptoxanthin 5′,6′-epoxide (**10**), β-cryptoxanthin 5,6,5′,6′-diepoxide (**11**), α-cryptoxanthin 5,6-epoxide (**12**) *etc*.) occur also in various higher plants.[7-9]



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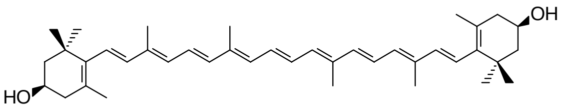

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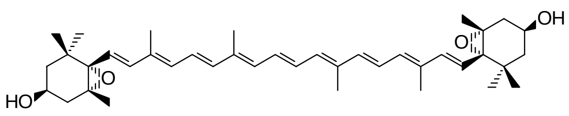

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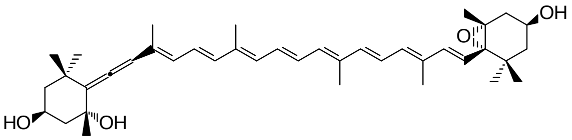

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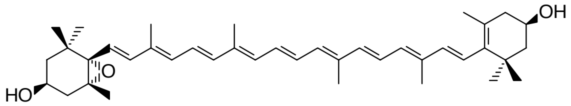

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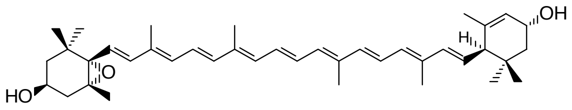

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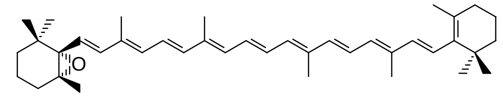

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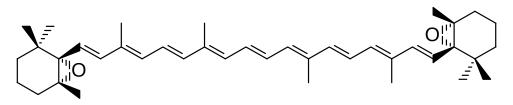

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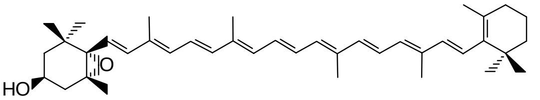

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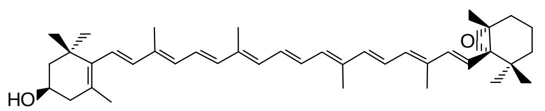

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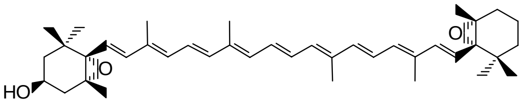

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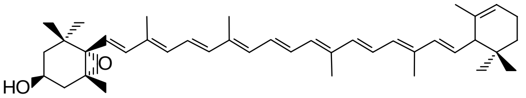

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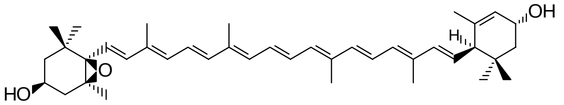
All natural xanthophyll 5,6-epoxides hitherto elucidated exhibit a *trans*-relationship between OH(3) and the epoxide group.[10] Thus, all naturally occurring 3-hydroxy-5,6-carotenoid epoxides have the 3*S*,5*R*,6*S* (*anti*) configuration. The *syn* (3*S*,5*S*,6*R*) epoxides can be prepared by the epoxidation with perbenzoic or perphthalic acid of carotenoids containing a β-ring. Depending on the applied epoxidizing reagent, different ratios of *syn*- and *anti*-epoxides were found. For example, the epoxidation of lutein (preferably the acetate form) with perbenzoic acid, gave two epoxides after deacetylation. The minor product (~5%) was identical with natural (*anti*)-lutein-epoxide (**6**), while the major product (~95%) had the 3*S*,5*S*,6*R*-configuration (*syn*-lutein-epoxide, **13**).[11] Epoxidation of zeaxanthin diacetate with perphthalic acid and subsequent deacetylation yielded 6% *anti*- (**5**) and 26% *syn*-antheraxanthin (**14**) as well as 4% *anti,anti*- (**3**), 35% *syn*,*anti*- (**15**) and 33% *syn*,*syn*-violaxanthin (**16**).[12] In the case of capsanthin the perphthalic acid mediated epoxidation resulted in the formation of (**17**) and (**18**) in 3 : 1 ratio.[13]



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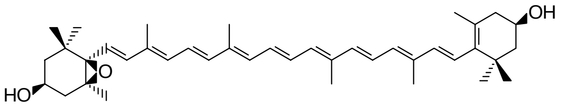

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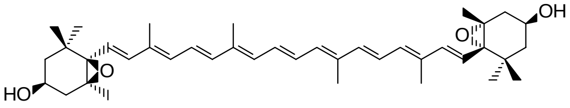

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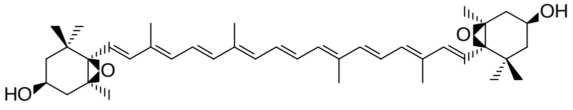

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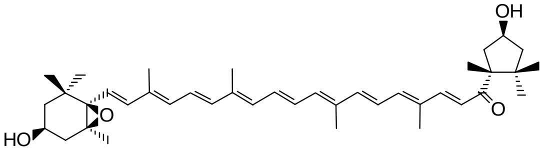

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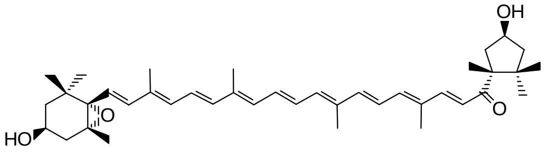

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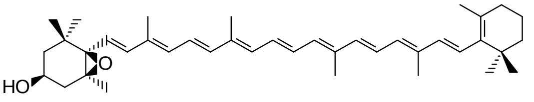
From the epoxidation mixture of β-cryptoxanthin-acetate, containing two differently substituted β-rings, we could isolate[14] the following epoxide derivatives: *anti*- (**9**) and *syn*- β-cryptoxanthin-5,6-epoxide (**19**), *anti*-β-cryptoxanthin-5′,6′-epoxide (**10**), *anti*,*anti*- (**11**) and *syn*,*syn*-β-cryptoxanthin-5,6,5,6′-diepoxide (**20**).



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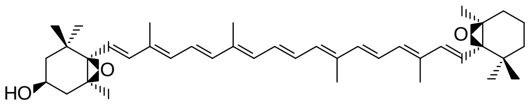

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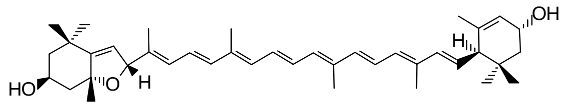
2. 5,8-Epoxy-carotenoids

5,8-Epoxy carotenoids (=furanoid oxides), together with their 5,6-epoxide isomers can be found in a variety of plants. However, it is by no means always certain whether they are natural pigments or artifacts formed from 5,6-epoxides via epoxide-furanoid rearrangement during the isolation process. 5,6-Epoxides readily rearrange to form 5,8-epoxides *in vitro* on treatment with dilute acids, on irradiation of the solutions, etc. According to the literature data, the amount of 5,8-epoxides, and their ratio to 5,6-epoxides, in plants vary widely. It seems, however, that in most cases the high furanoid oxide content is connected to the drying and extraction procedures of the analysed sample.[15]

During the epoxide-furanoid rearrangement, the configuration at C(5) remains unchanged and, until today, there are no exceptions to this rule. The HPLC analysis of natural 5,8-epoxide containing samples proved the presence of both C(8)-epimers in these samples. Thus, one can find reports on the coexistence of flavoxanthin (3*S*,5*R*,8*R*, **19**) and chrysantemaxanthin (3*S*,5*R*,8*S*, **20**),[16] mutatoxanthin epimers (3*S*,5*R*,8*R*, **21** and 3*S*,5*R*,8*S*, **22**) in red paprika (*Capsicum annuum*)[17] as well as of the luteoxanthin epimeric pair (3*S*,5*R*,8*R*, **23** and 3*S*,5*R*,8*S*, **24**) and the three epimers of auroxanthin (3*S*,5*R*,8*R*,3′*S*,5′*R*,8′*R*, **25,** 3*S*,5*R*,8*S*,3′*S*,5′*R*,8′*S*, **26** and 3*S*,5*R*,8*R*, 3′*S*,5′*R*,8′*S*, **27**) in *Rosa foetida*.[18]



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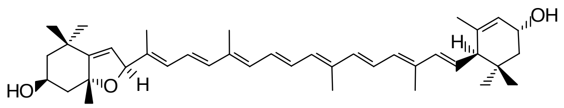

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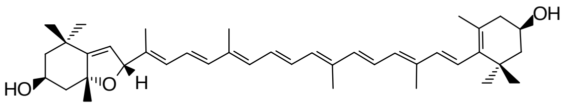

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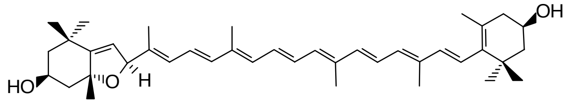

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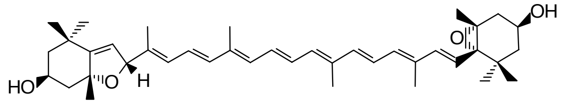

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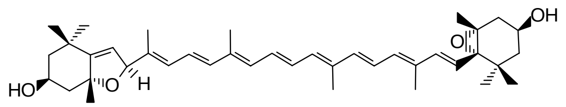

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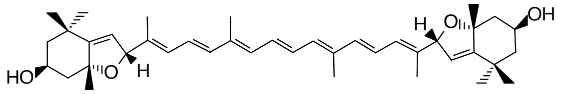

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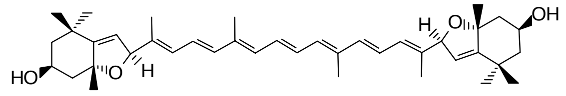

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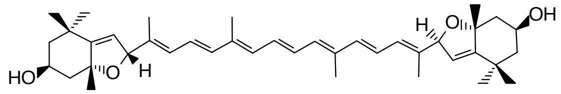

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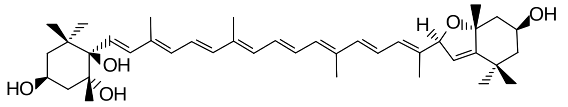
Two new polar carotenoids that contained the 3,5,6-trihydroxy-β end group, namely latochrome epimers (**28**, **29**), also were isolated from the petals of a yellow rose.[18] The furanoid isomers of neoxanthin, the neochrome epimers (**30**, **31**), were isolated from hips of *Rosa pomifera*.[19]



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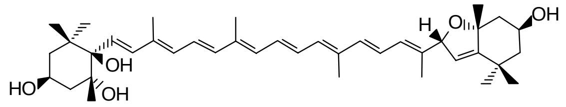

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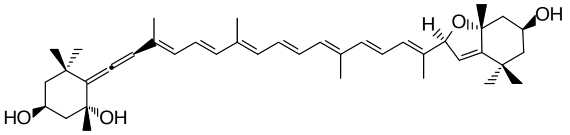

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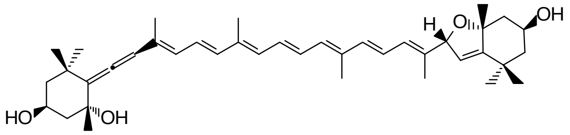

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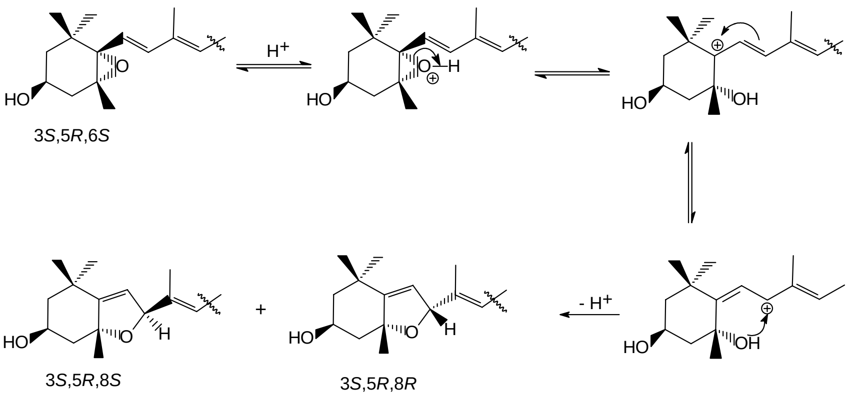
The 5,6-epoxides of cyclic carotenoids are readily rearranged into 5,8-epoxides in the presence of acid catalysts. The mechanism of the epoxide-furanoid rearrangement was proposed by Eugster (Scheme 1).[20]

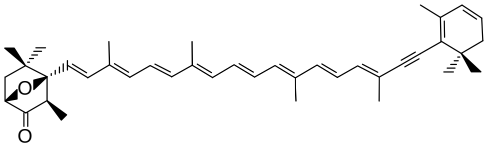
The reaction leads to a shortening of the chromophore by one conjugated double bond, causing a characteristic hypsochromic shift of the UV/VIS spectrum of *ca*. 20 nm for each 5,6-epoxy group. This chemical reaction is the most useful microscale test for the fast identification of carotenoid 5,6-epoxides.



Scheme 1

3. 3,6-Epoxy-carotenoids

Another new type of epoxide is represented by carotenoids with the 3,6-epoxy-3,6-dihydro- β (7-oxabicyclo[2.2.1]heptane) end group, such as eutreptiellanone (**32**), α-cryptoeutreptiellanone (**33**) and β-cryptoeutreptiellanone (**34**), which were isolated for the first time from the marine alga *Eutreptiella gymnastica*.[21-23]



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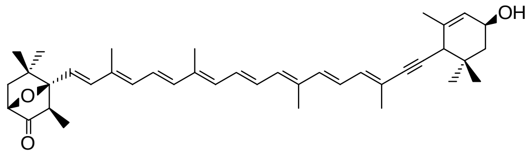

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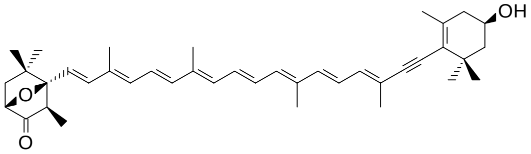

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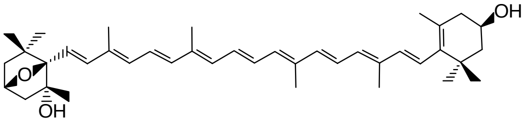
The isolation of cucurbitaxanthin A (**35**) and cucurbitaxanthin B (**36**) from pumpkin,[24] and of cucurbitaxanthin A (**35**) and capsanthin 3,6-epoxide (**37**) from red paprika[25] was successful at about the same time. In 1990, Eugster and Gmünder[26] published an attempt to synthesise the cycloviolaxanthin (**38**) containing two 5-hydroxy-3,6-epoxy groups. Unfortunately, the last reaction step resulted in a compound with heterocyclic rings, instead of cycloviolaxanthin. At the same time, cycloviolaxanthin (**38**) and cucurbitaxanthin B (**36**) could be isolated in our laboratory from red paprika in very small amounts.[27]



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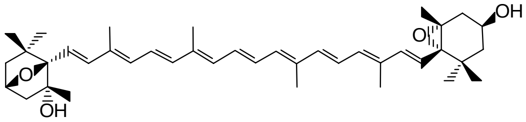

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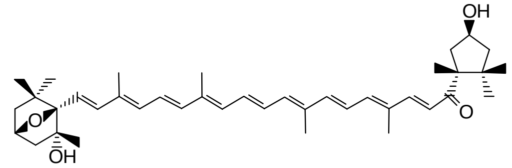

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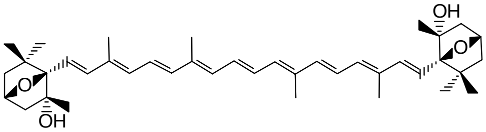

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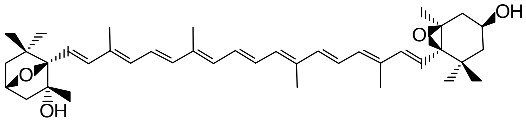
The preparation of semisynthetic *anti*- (**36**) and *syn*-cucurbitaxanthin B (**39**) was published in 1993.[28] Cucurbitaxanthin A (**35**) was converted into the trimethylsilyl ether and then epoxidized with monoperphthalic acid. After deprotection and chromatographic separation, cucurbitaxanthin B (**36**) and 5',6'-diepicucurbitaxanthin B (**39**) were obtained.

A few years later, a new isolation method for carotenoid 3,6-epoxides from red paprika was developed.[29] This method, in essence, was based on solvent distribution and fractionated precipitation. The fresh red paprika was first extracted with methanol, then with diethyl ether. After saponification, the methanolic fraction contained the target carotenoids, besides a number of different secondary compounds, which could be separated mostly by solvent partition and sequential precipitation steps. This way cucurbitaxanthin A (**35**) and B (**36**), cucurbitachrome 3'*S*,5'*R*,8'*S*- (**40**) and 3'*S*,5'*R*,8'*R*-epimers (**41**), and capsanthin 3,6-epoxide (**37**) were isolated. In addition, 3,6-epoxy-5,6,5′,6′-tetrahydro-β,β-carotene-5,3′,5′,6′-tetrol (**42**) was isolated in a very small amount and identified tentatively.



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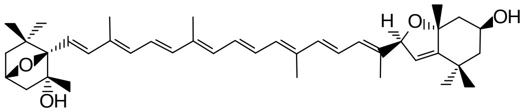

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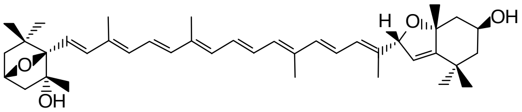

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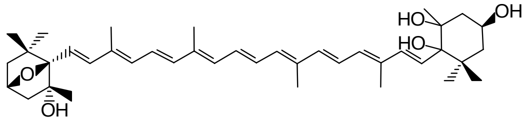
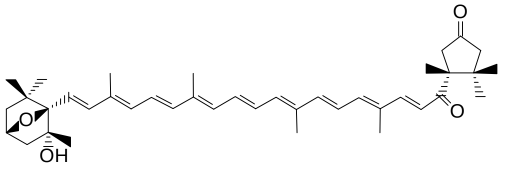


42

In 1998, Hornero-Mendez and Mínguez-Mosquera[30] isolated capsolutein from red paprika and characterized it by mass spectrometry as cucurbitaxanthin A (**35**). Maoka et al.[31] described another new 3,6-epoxy-carotenoid, namely the capsanthone 3,6-epoxide (**43**), isolated from red paprika (*Capsicum annuum* L).



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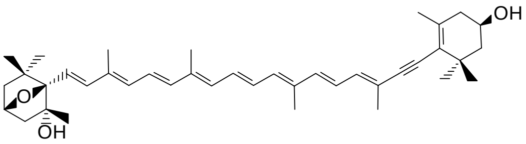

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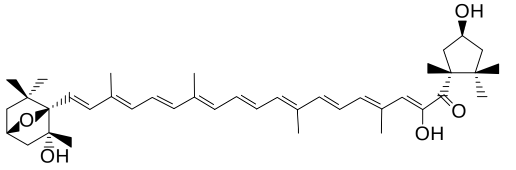

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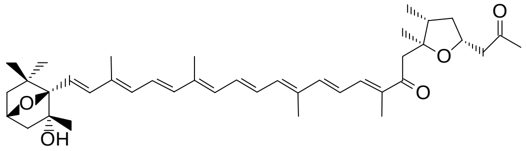
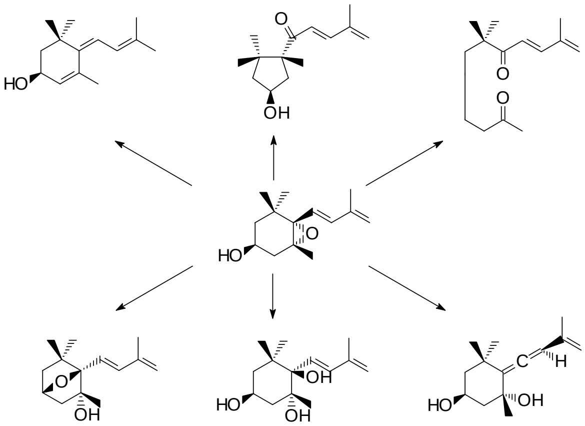


46

Another three 3,6-epoxy-carotenoids with very interesting end-groups (7′,8′-didehydro-3-hydroxy-β: **44**, 8′-hydroxy-6′-oxo-κ: **45**, and 3′,6′-epoxy-1′,2′,5’,6′,7′,8′ hexahydro-6′-methyl-16′-nor-1′8′-dioxo-φ-end group: **46**) were isolated also by the same group[32] from the oyster *Crassostrea gigas*,

4. Transformation of 5,6-epoxy-carotenoids

The number of naturally-occurring carotenoids whose structures have been elucidated now numbers more than 600. However, these carotenoids show a wide diversity of structural features and modifications to the basic end-groups, and virtually nothing is known about the biosynthetic reactions that lead to the formation of these structures. It has been known for a long time that the 5,6-epoxy structure can give rise to a wide variety of carotenoid end-groups and Scheme 2 summarizes the plausible biosynthetic transformations of the 5,6-epoxy-β-ring (with or without a 3-hydroxy group) to give diverse structures such as the cyclopentane (κ-ring) carotenoids capsanthin and capsorubin, the *retro*-carotenoids eschscholtzxanthin and rhodoxanthin, allenic carotenoids (eg. neoxanthin, fucoxanthin), 5,6-diols (eg. karpoxanthin, latoxanthin), 3,6-epoxides as in eutreptiellanone and cucurbitaxanthin, the 6-hydroxy-γ-ring structure found in prasinoxanthin and nigroxanthin, as well as the 5,6-seco-5,6-dione-end-group of semi-β-carotenone.[33]

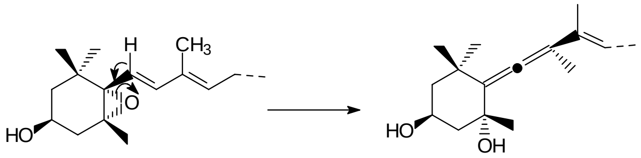


Scheme 2

Although these proposed routes are, on paper, very reasonable, in general there is no experimental evidence to support them. The intermediate steps in the carotenoid biosynthetic pathway were postulated several decades ago by standard biochemical analyses using labeled precursors, specific inhibitors and characterization of mutants. In recent years, the molecular-genetic approach to carotenogenesis study has provided a wealth of information and new perspectives of both the enzyme activities and the regulation of the pathway.[34]

4.1. Formation of the allenic end-group

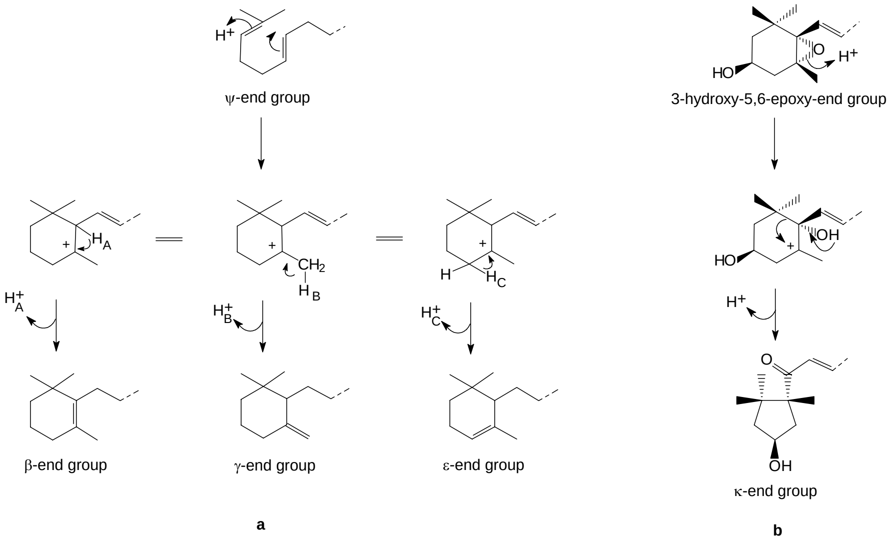
As shown in Scheme 3 the allenic group originates from proton abstraction from C(7), neighbouring one of the 5,6-epoxy-5,6-dihydro-β rings of violaxanthin, followed by rearrangement of the epoxy group to the 5-hydroxy-allenic end group.[35] This reaction is catalysed by the enzyme neoxanthin synthase isolated and characterized in 2000.[36]



Scheme 3

4.2. Formation of the κ-end-group

The proposal that the κ -end group is formed as shown in Scheme 4 has received support from experiments that have demonstrated the incorporation of radioactive antheraxanthin and violaxanthin into capsanthin and capsorubin, respectively, by chromoplasts of *Capsicum annuum*.[37] The capsanthin-capsorubin synthase (CCS) enzyme that catalyses the conversion of 5,6-epoxy-end groups into κ-end groups was isolated and characterized in 1994.[38] Certain similarities of CCS were observed with the *C. annuum* lycopene cyclase, the enzyme catalyzing the cyclization of lycopene.[39]

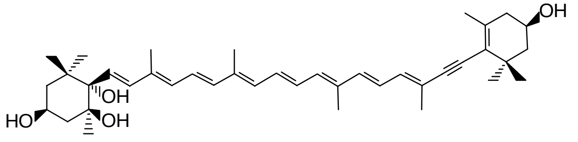


Scheme 4

The fact that CCS also exhibits lycopene cyclase activity, is likely to be related to similarities in the chemical mechanisms leading to the formation of β-rings in β-carotene (Scheme 4a) and κ-rings in capsanthin and capsorubin (Scheme 4b). In both mechanisms, an intermediate carbenium ion at C(5) forms[40] and, in addition, both reactions probably are initiated by protonic attack on either a double bond or an epoxy group.

4.3. Ring opening reaction of the 3-hydroxy-5,6-epoxy group

Another interesting transformation of the 3-hydroxy-5,6-epoxy-carotenoids is the ring opening reaction, which results in 3,5,6-trihydroxy compounds. Naturally occurring carotenoids with the 3,5,6-trihydroxy-5,6-dihydro-β-end group have been found in several different sources. Heteroxanthin (**47**) was isolated from *Euglena gracilis,*[41] karpoxanthin (**48**) from ripe hips of *Rosa pomifera*[42], karpoxanthin (**48**) and 6-epikarpoxanthin (**49**) from petals and pollen of *Lilium tigrinum,*[43] latoxanthin (**50**) from petals of *Rosa foetida,*[18] and neoflor (**51**) and 6-epineoflor (**52**) from petals of *Trollius europaeus*[44].



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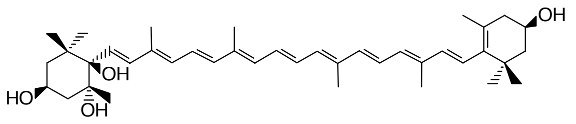

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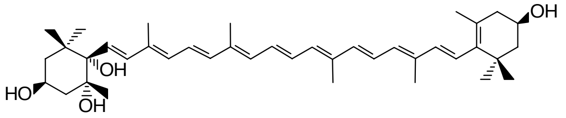

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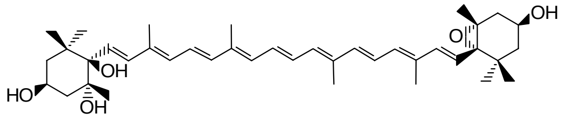

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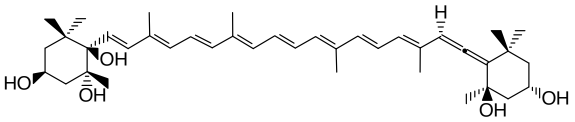

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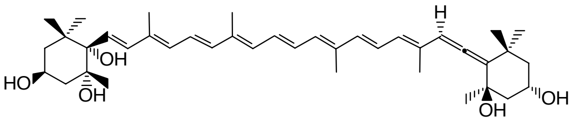

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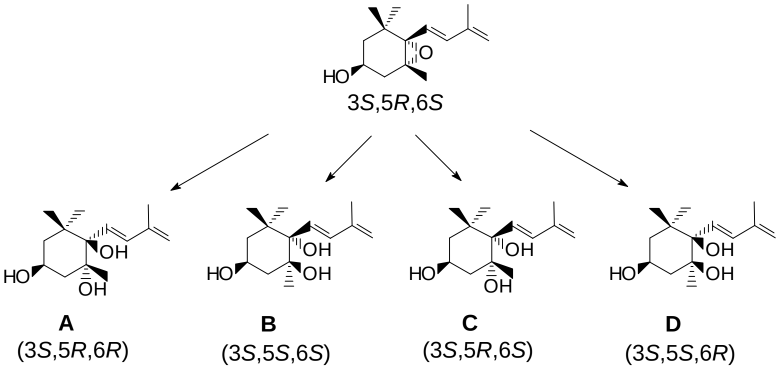
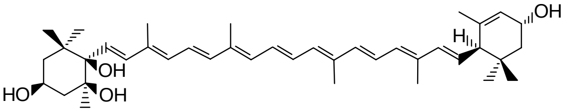
The structural elucidation of the 3,5,6-trihydroxy-β-end group was performed by Eugster.[16,45] Formally, four end-groups (**A**-**D**) can be formed from the (3*S*,5*R*,6*S*)-3-hydroxy-5,6-epoxy-β-end group (Scheme 5).



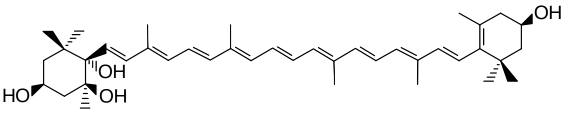
Scheme 5

Several ionone derivatives and carotenoids bearing this end group were prepared by partial synthesis, and spectroscopic data for the 3,5,6-trihydroxy-5,6-dihydro-β-end group with different configurations [(3*S*,5*R*,6*R*) (**A**), (3*S*,5*S*,6*S*) **(B)**, (3*S*,5*R*,6*S*) (**C**), and (3*S*,5*S*,6*R*) (**D**)] have been established. For the structural elucidation of the 3,5,6-trihydroxy-5,6-dihydro-β-end groups, acid-catalysed partial hydrolysis of 3-hydroxy-5,6-epoxy carotenoids was used successfully by Eugster and co-workers.[16,42,44] In this way were obtained, karpoxanthin (**48**) and 6-epikarpoxanthin (**49**) from antheraxanthin (**5**),[42] neoflor (**51**) and 6-epineoflor (**52**) from neoxanthin (**4**),[44] and only one compound, (3*S*,5*S*,6*R*3’*R*,6’*R*)-β,ε-carotene-3,5,6,3’-tetrol (**53**), from *syn*-lutein 5,6-epoxide (**13**).[16]



53

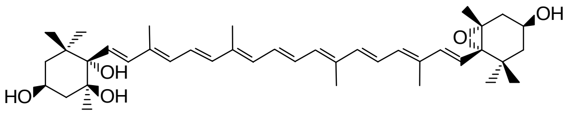
These reactions demonstrated that, during the hydrolysis of 5,6-epoxy carotenoids, the configuration at C(5) is maintained, whereas at C(6) both configurations are obtained.[16,45]
Based on these results, the configuration of the 3,5,6-trihydroxy-5,6-dihydro-β-end group could be determined as 3*S*,5*R*,6*R* (**A**) in karpoxanthin (**48**) originating from ripe hips of *Rosa pomifera,*[42] latoxanthin (**50**) originating from *Rosa foetida,*[18] and neoflor (**51**) originating from *Trollius europaeus,*[44] and as 3*S*,5*R*,6*S* (**C**) in 6-epikarpoxanthin (**49**) originating from ripe hips of *Rosa pomifera*[42] and 6-epineoflor (**52**) originating from *Trollius europaeus*.[44]
On the other hand, it is of great interest that for karpoxanthin isolated from red paprika, the 3*S*,5*S*,6*S*-configuration (**B**, **54**) was established.[46]



54

Twelve years later, as a continuation of our work on paprika carotenoids, four carotenoids (**54**, **49, 55** and **56)** bearing the 3,5,6-trihydroxy-β-end group were isolated from red spice paprika (*C.a*. var. *longum*).[47] The structures of these compounds were unequivocally assigned from their spectroscopic data and compared to literature data. Since **54** ((all-*E*,3*S*,5*S*,6*S*,3'*R*)-5,6-dihydro-β,β-carotene-3,5,6,3'-tetrol) is a diastereoisomer of karpoxanthin (**48**) with reversed configuration at C(5) and C(6), we proposed the trivial name 5,6-diepikarpoxanthin for **54**. Similarly, since **55**, identified as (all-*E*,3*S*,5*S*,6*S*,3'*S*,5'*R*,6'*S*)-5',6'-epoxy-5,6,5',6'-dihydro-β,β-carotene-3,5,6,3'-tetrol, is a 5,6-diastereomer of latoxanthin (**50**), the name 5,6-diepilatoxanthin was proposed. The carotenoid **56**, containing a κ-end group as second end group, represented a carotenoid with an unknown constitution. **56** was identified as (all-*E*,3*S*,5*S*,6*S*,3'*S*,5'*R*)-5,6-

dihydro-3,5,6,3'-tetrahydroxy-β,κ-caroten-6'-one, and, by analogy with **54** and **55**, the name 5,6-diepicapsokarpoxanthin was proposed for it.



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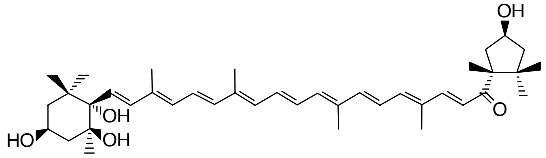
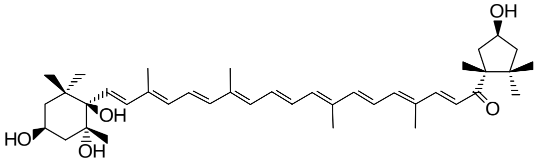


56

For the exact characterization of the configuration of 3,5,6-trihydroxy-β-end groups and comparative studies, the hydrolysis of *anti*- and *syn*-capsanthin 5,6-epoxides (**18**, **17**) and antheraxanthins (**5**, **14**) was performed in our laboratory.[48,49] The hydrolysis of *anti*- (3*S*,5*R*,6*S*)-5,6-epoxy-carotenoids resulted in the formation of two 3,5,6-trihydroxy-carotenoids with the (3*S*,5*R*,6*R*)=**A** (capsokarpoxanthin: **57**, karpoxanthin: **48**) and (3*S*,5*R*,6*S*)=**C** (6-epicapsokarpoxanthin: **58**, 6-epikarpoxanthin: **49**) configurations. The hydrolysis of *syn*-(3*S*,5*S*,6*R*)-5,6-epoxy-carotenoids gave only one 3,5,6-trihydroxy-compound with the (3*S*,5*S*,6*R*)=**D** (5-epicapsokarpoxanthin: **59**, 5-epikarpoxanthin: **60**) configuration. Carotenoids with the (3*S*,5*S*,6*S*)-configuration (5,6-diepi-compounds with **B** end group) were not formed in this way.



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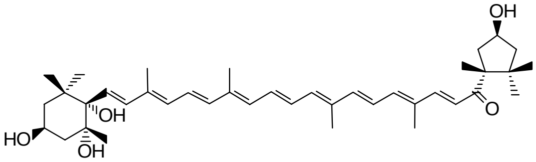

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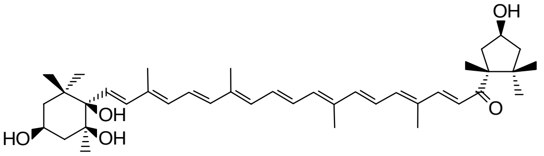

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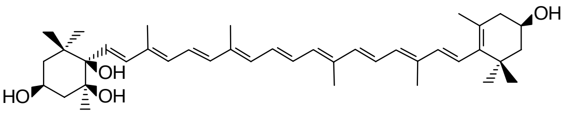

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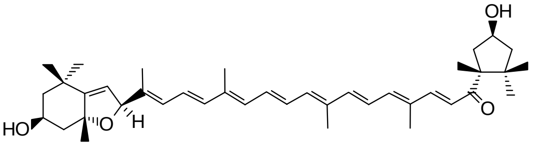
The appropriate 5,8-furanoid oxides, namely capsochromes (**61**-**64**) and mutatoxanthins (**21**, **22**, **65**, **66**), also were isolated as main products of these reactions.



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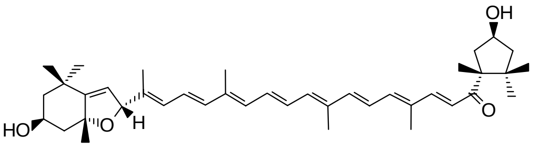

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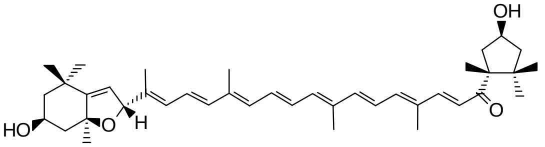

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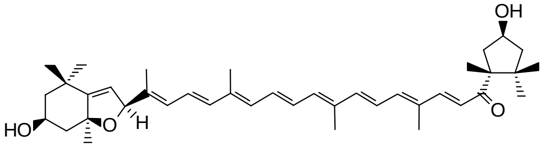

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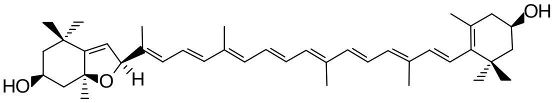

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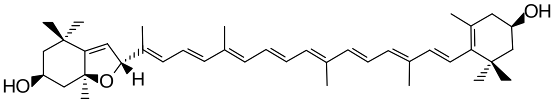

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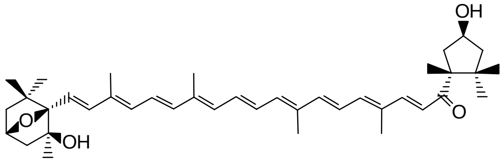
As minor compounds, (3*S*,5*R*,6*R*)-capsanthin 3,6-epoxide (**43**) and cucurbitaxanthin A (**35**), were detected from *anti*-5,6-epoxides, while from *syn*-5,6-epoxides, 5-epicapsanthin 3,6-epoxide (**67**) and 5-epicucurbitaxanthin A (**68**) were obtained. This was the first published work regarding the semisynthesis of 3,6-epoxy-carotenoids.[48,49]



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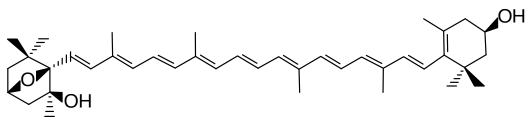
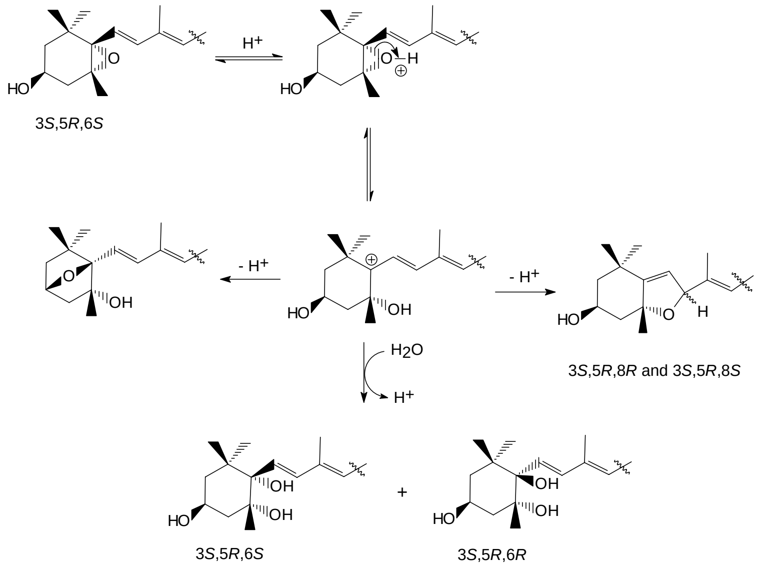


68

Our investigations confirmed the results of Eugster and co-workers[42,45] and showed that the configuration of 3,5,6-trihydroxy carotenoids originating from 3-hydroxy-5,6-epoxy-carotenoids by hydrolysis is independent of the remote end group. The same two (3*S*,5*R*,6*S*)- and (3*S*,5*R*,6*R*)-3,5,6-trihydroxy-β-end (**C** and **A**) groups have been obtained from (3*S*,5*R*,6*S*)-antheraxanthin (**5**) containing the 3-hydroxy-β-end group, from (3*S*,5*R*,6*S*)-lutein 5,6-epoxide (**6**) containing the 3-hydroxy-ε-end group, and from (3*S*,5*R*,6*S*)-capsanthin 5,6-epoxide (**18**) containing the 3-hydroxy-κ-end group. The (3*S*,5*S*,6*R*)-trihydroxy-β-end (**D**) group has been obtained from (3*S*,5*S*,6*R*)-antheraxanthin (**14**), from (3*S*,5*S*,6*R*)-lutein 5,6-epoxide (**13**) and from (3*S*,5*S*,6*R*)-capsanthin 5,6-epoxide (**17**). In the acid-catalysed hydrolysis, the configuration of the 3-hydroxy-5,6-epoxy-β-end group is decisive for the configuration of the 3,5,6-trihydroxy-β-end group. The proposed mechanism for the formation of 3,5,6-trihydroxy-carotenoids[48] is shown in Scheme 6. The acid-catalysed hydrolysis of the (3*S*,5*R*,6*S*)-5,6-epoxy-β-end group results in a mixture of compounds with the (3*S*,5*R*,6*R*)- and the (3*S*,5*R*,6*S*)-end groups, i.e. with retention of configuration at C(5). This can be explained by the formation of a carbenium ion at C(6).



Scheme 6

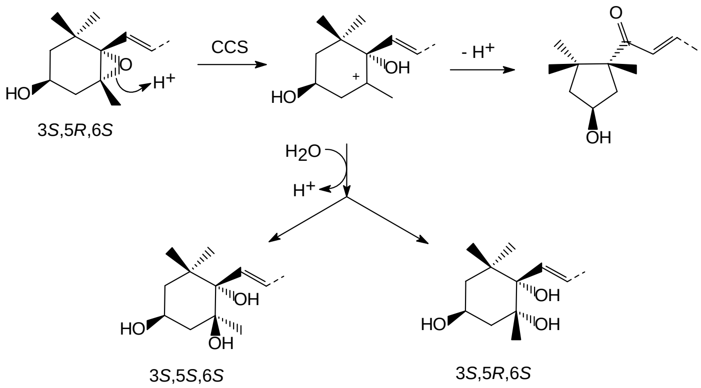
The proposed mechanism also involves the formation of 3,6-epoxy-end group. The nucleophilic attack of the 3-hydroxy group to C(6) results in the 3,6-epoxy-5-hydroxy-β-end group.

The isolation of 5,6-diepi-compounds demonstrated that the chirality of karpoxanthin and latoxanthin differed depending on the natural sources. In paprika, for example, the (3*S*,5*S*,6*S*)=**B** configuration for the 3,5,6-trihydroxy-5,6-dihydro-β-end group (**54** and **55**) can be found, while other organisms contain the (3*S*,5*R*,6*R*)=**A** configuration (**48** and **50**). In addition, the first capsokarpoxanthin isolated from natural sources was **56**, possessing the (3*S*,5*S*,6*S*=**B**) configuration.

It can be assumed that, in Nature, the carotenoids with the 3,5,6-trihydroxy-β-end group are formed from the 3-hydroxy-5,6-epoxy-β-end group, the (3*S*,5*R*,6*S*)-3-hydroxy-5,6-epoxy-β-end group of violaxanthin being a possible precursor in all organisms. However, it is highly probable that in paprika, which contains carotenoids with κ-end group(s), compared to other organisms, the 3,5,6-trihydroxy-β-end group is formed from the corresponding 3-hydroxy-5,6-epoxy-β-end group following a different mechanism. This assumption was confirmed when 5,6-diepikarpoxanthin (**54**), 6-epikarpoxanthin (**49**) and 5,6-diepicapsokarpoxanthin (**56**), as minor compounds, were isolated from the petals of *Lilium tigrinum*, which also contains capsanthin and capsorubin, carotenoids with κ-end groups.[50]

We supposed that the formation of a mixture of (3*S*,5*S*,6*S*)- (**B**) and (3*S*,5*R*,6*S*)-3,5,6-trihydroxy-β-end (**C**) groups from the (3*S*,5*R*,6*S*)-3-hydroxy-5,6-epoxy-end group could be explained by the formation of a carbenium ion at C(5) (Scheme 7).[47]

The formation of a carbenium ion at C(5) as intermediate has been proposed in the mechanism for the conversion of antheraxanthin (**5**) and violaxanthin (**3**) into the κ-rings of capsanthin and capsorubin, the most important carotenoids in paprika.[39] As shown in Scheme 7, the mechanism involves a pinacolic rearrangement (also see Scheme 4).



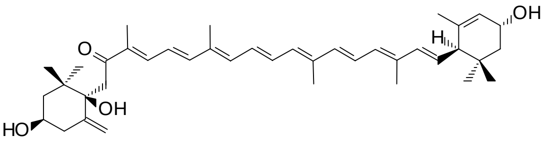
Scheme 7

On the basis of this suggested reaction mechanism, either the (3*S*,5*S*,6*S*)- (**B**) or the (3*S*,5*R*,6*S*)-end (**C**) group may be formed *via* the carbenium ion at C(5). Therefore, during the enzyme-catalysed hydrolysis of carotenoid-5,6-epoxides, the configuration at C(5) may change, but the configuration at C(6) remains unchanged.

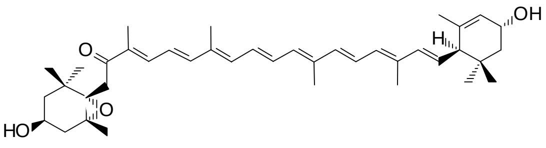
In the plants which do not contain carotenoids with the κ-end group (e.g. *Rosa foetida*, ripe hips of *Rosa pomifera*), the ring opening of carotenoid-5,6-epoxides probably is acid catalysed and follows the mechanism given in Scheme 6. During the acid-catalysed hydrolysis of carotenoid-5,6-epoxides, the configuration at C(6) may change, whereas that at C(5) remains unchanged. The different biosynthetic routes may explain the differences between the configurations of carotenoids with the 3,5,6-trihydroxy-β-end group isolated from different sources.

4.4. Formation of the γ-end group

Liaaen-Jensen and co-workers isolated prasinoxanthin[51] (**69**) and preprasinoxanthin[52] (**70**) from *Prasinophyceae*.

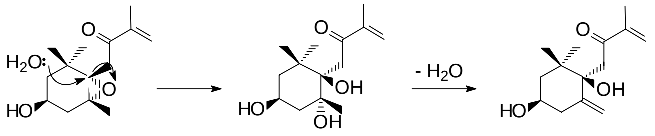


69



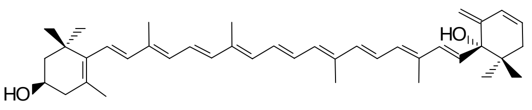
70

The unique γ-end group of prasinoxanthin (**69**) was suspected to be formed biosynthetically from the precursor, preprasinoxanthin (**70**), as is shown in Scheme 8.[52]

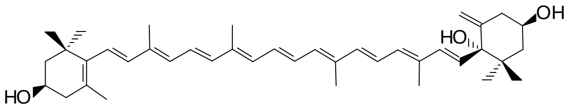


Scheme 8

In our laboratory, during the isolation of cycloviolaxanthin[27] from paprika, several unknown carotenoids were obtained by column chromatography. Further investigation of the chromatographic zone on the $CaCO_3$ column between cucurbitaxanthin A and B furnished a new carotenoid (**71**) for which the name nigroxanthin was proposed. Nigroxanthin was isolated and identified as (all-*E*)-3',4'-didehydro-β,γ-carotene-3,6'-diol.[53] The configuration at C(6') at that time was unknown. Several years later, another carotenoid containing the γ-end group, namely the prenigroxanthin (**72**), was isolated from red spice paprika (*C.a.* var. *longum*). The constitution of this carotenoid was identified as β,γ-carotene-3,3',6'-triol.[54]

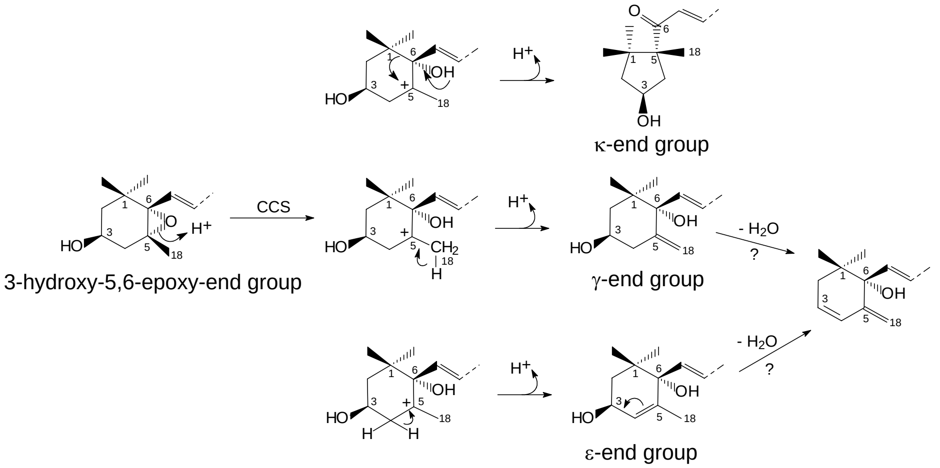


71



72

As the configuration at C(6') of **71** and **72** had not yet been clarified by modern spectroscopic methods, the biosynthetic pathway of paprika carotenoids was taken into consideration. Compounds **71** and **72** may be formed from antheraxanthin (**5**) and their occurrence is interrelated with the biosynthesis of the κ-end group. The probable biosynthetic route for the formation of the γ-end group is as follows: enzymatic opening of the 5,6-epoxy ring results in a carbenium ion at C(5) in compliance with Scheme 9. According to three different routes, this intermediate can be stabilized by the formation of: a) a κ-end group ; b) a 3,6-dihydroxy-γ-end group or c) a 3,6-dihydroxy-ε-end group. It was assumed that the latter end group was not stable and it could be rearranged easily into the 6-hydroxy-3,4-didehydro-ε-end group by elimination of water. In these reactions, the configuration at C(6) remains unchanged, thus, strongly supporting the proposed structures with the 6'*S* configuration for nigroxanthin (**71**) and prenigroxanthin (**72**).



Scheme 9

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