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In Vitro and *In Vivo* Transformations of Lutein

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Abstract: Lutein ((all-*E*,3*R*,3'*R*,6'*R*)-β,ε-carotene-3,3'-diol) is the main carotenoid that can be found in light-harvesting complexes of higher plants and it is also involved in human diet. In the extracts of human plasma the 3'-epimer of lutein, several geometrical isomers, dehydration and oxidation products of lutein have been identified. It was also established that lutein reduces the risk of AMD (age-related macular degeneration). This mini review focuses on the *in vitro* and *in vivo* transformations of lutein.

Key Words: Lutein, anhydroluteins, 3'-epilutein, 3-oxolutein.

1. INTRODUCTION

Carotenoids belong to the group of yellow or red pigments that occur widely in plants, animals and humans. They are synthesized by plants and by some microorganisms and are introduced into human and animal organisms exclusively with diet. These higher organisms are incapable of the *de novo* synthesis of carotenoids, but sometimes capable of their structural modification. Carotenoids have high physiological importance and fulfill many tasks. Primarily, they always accompany chlorophyll and assist photosynthesis and phototaxis as auxiliary light absorbers or, on the other hand, protect plants and microorganisms against excessive irradiation. Furthermore, they strongly interact with reactive oxygen species thus act as potent free radical traps, singlet oxygen scavengers and lipid antioxidants (some of them are precursors of vitamin A) in plants and animals. In addition to this physiological and medical importance changes in the contents and structure of carotenoids can also act as markers of environmental damage.

The skeleton of carotenoids is usually composed of eight isoprene units joined head-to-tail, except the "middle" of the molecule where a tail-to-tail connection can be observed. The terminal isoprene units form open chain or cyclic structures called end groups. Carotenoids contain long, aliphatic, conjugated double bond systems, i.e. polyenes. The majority of natural carotenoids have double bonds in all-*trans* position, only a few of them show *cis-trans* configuration.

A part of carotenoids are hydrocarbons with the molecular formula $C_{40}H_{56}$. These hydrocarbons are called carotenes. The other part of carotenoids are the oxygenated derivatives of carotenes with various combinations of hydroxy, epoxy, aldehyde, keto, lactone, carboxylic acid, ester and/or phenolic functions. These compounds are called xanthophylls and the oxygen-containing functional groups are always located at the end groups, not within the multi-conjugated system. No heteroatom other than oxygen has so far been known to be present in natural carotenoids [1].

Lutein ((all-*E*,3*R*,3'*R*,6'*R*)-β,ε-carotene-3,3'-diol) (**1**, Fig. **1**) is the main xanthophyll in the major light-harvesting pigment-protein complex of higher plants and is involved in energy-transfer mechanisms during photosynthesis. In fatty acid ester form lutein is widely distributed in flowers, yellow autumn leaves, fruits and vegetables [2], and in this way it is also involved in human diet. In the extracts of human plasma the 3'-epimer of lutein (3'-epilutein), several geometrical isomers, dehydration (anhydrolutein I and II) and oxidation products (3'-oxo-lutein) of lutein have been identified. It was also established that lutein reduces the risk of AMD (age-related macular degeneration) [3].

The correct molecular formula $C_{40}H_{56}O_2$ for lutein isolated from green leaves was established first in the laboratory of Wilstatter in 1907 [4] by accurate combustion analysis, together with classical molecular weight determination. The total synthesis of lutein was published in 1980 by Mayer and Rüttimann [5]. Confirmation of the constitution and absolute configuration by X-ray crystal structure analyses was described in 2004 [6].

Lutein can exist in eight stereoisomeric forms as a result of the presence of three stereocenters at 3, 3', and 6' carbon atoms. In addition, the hydroxyl group at carbon 3' of lutein is allylic.

The 3'-epimer of lutein with (all-*E*,3*R*,3'*S*,6'*R*) configuration is called 3'-epilutein (lutein B, Calthaxanthin) (**2**) and was isolated from the flowers of Marsh Marigold (*Caltha palustris*) [7, 8] and from goldfish (*Carassius auratus*), together with α-doradexanthin ((all-*E*,3*S*,3'*S*,6'*R*)-3,3'-dihydroxy-β,ε-caroten-4-one) (**3**) [9]. Compound (**2**) was also detected in the anthers of flowers such as *Rosa gallica 'officinalis'* and *Paeonia officinalis* [10]. It was established later that 3'-epilutein and α-doradexanthin are also widespread in the animal kingdom [11].

The 6'-epilutein (lutein F, (all-*E*,3*R*,3'*R*,6'*S*)-β,ε-carotene-3,3'-diol) (**4**) and the 3',6'-diepilutein (lutein D, (all-*E*,3*R*,3'*S*,6'*S*)-β,ε-carotene-3,3'-diol) (**5**) were isolated as minor carotenoids from the eggs of dolphin fish (1-3% of total) and of flying fish (up to 0.4% of total) [12]. The 3,6'-epilutein (lutein G, (all-*E*,3*S*,3'*R*,6'*S*)-β,ε-carotene-3,3'-diol) (**6**) together with lutein F (**4**) were isolated from marine fish (*Podothecus sachi* and *Epinephelus fario*) integuments [11]. While the principal isomer of lutein with (3*R*,3'*R*,6'*R*) configuration occurs mainly in plants, the other stereoisomers seem to be typical of animal carotenoids.

2. *IN VITRO* TRANSFORMATIONS OF LUTEIN

2.1. Dehydration Reaction of Lutein

Anhydroluteins, the dehydration products of lutein (**1**), have been known for a long time [13-15] as semisynthetic derivatives of lutein. Zechmeister and his co-workers prepared the first anhydroluteins [13]. Lutein was mixed with naphthalene (in order to depress the melting point), melted in the presence of fused boric acid, and then kept at 140 °C for a few minutes. The three main products obtained were named as deoxylutein I (or anhydrolutein III) (**7**, Fig. **2**), deoxylutein II (or anhydrolutein I) (**8**) and deoxylutein III (or anhydrolutein II) (**9**), however, their proposed constitutions were not confirmed and their stereochemistry were also not clarified in this work. Baranyai *et al.* [16] reinvestigated the structure of deoxyluteins in 1984. They prepared the anhydrolutein compounds by Zechmeister's original procedure and investigated their structures by ^{1}H-, ^{13}C-NMR and circular dichroism (CD). The results proved the earlier tentative constitutions and led to the assignment of the absolute configurations of the asymmetry centres. Under the employed conditions the yields based on lutein were: anhydrolutein I (**8**) 10%, anhydrolutein II (**9**) 4% and anhydrolutein III (**7**) 3-4%.

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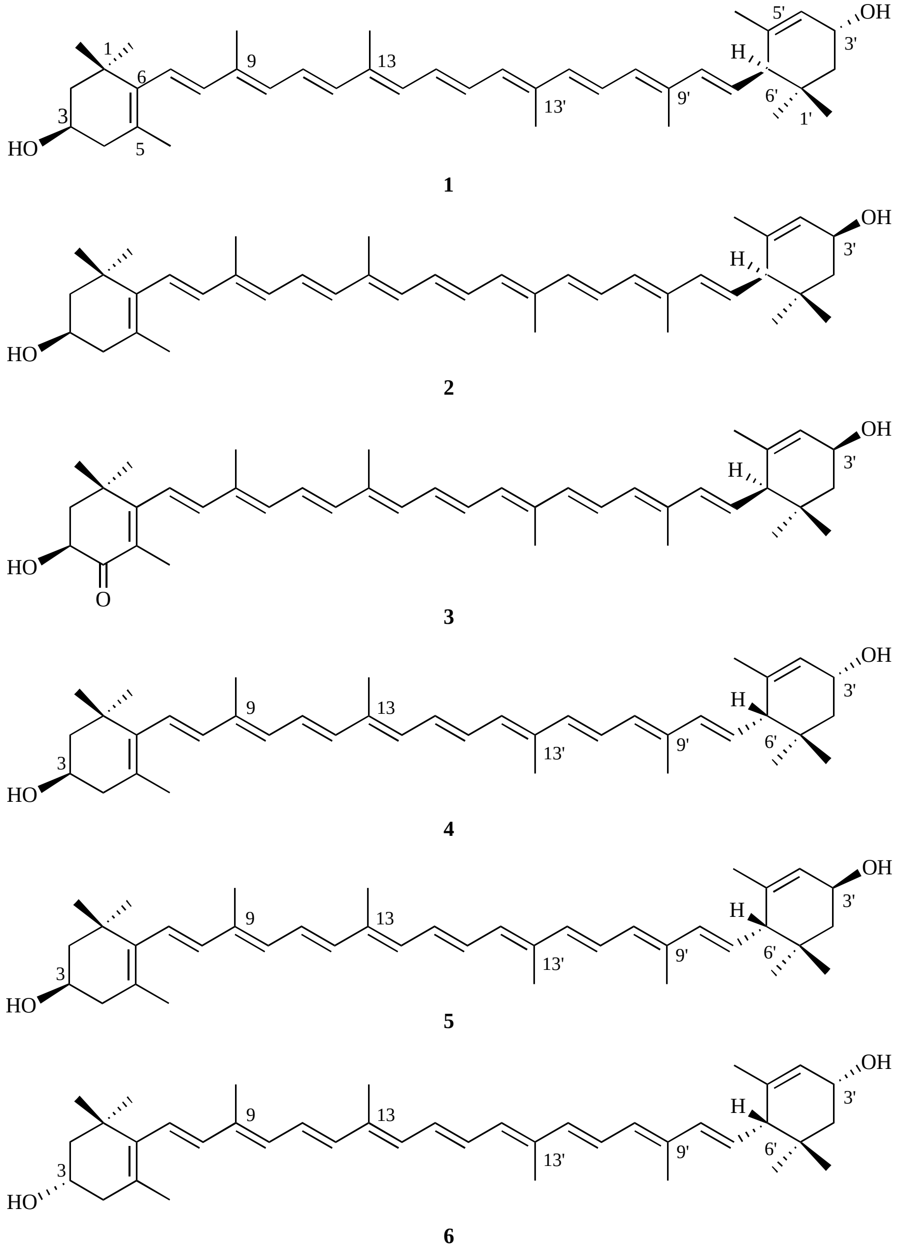

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Fig. (1).

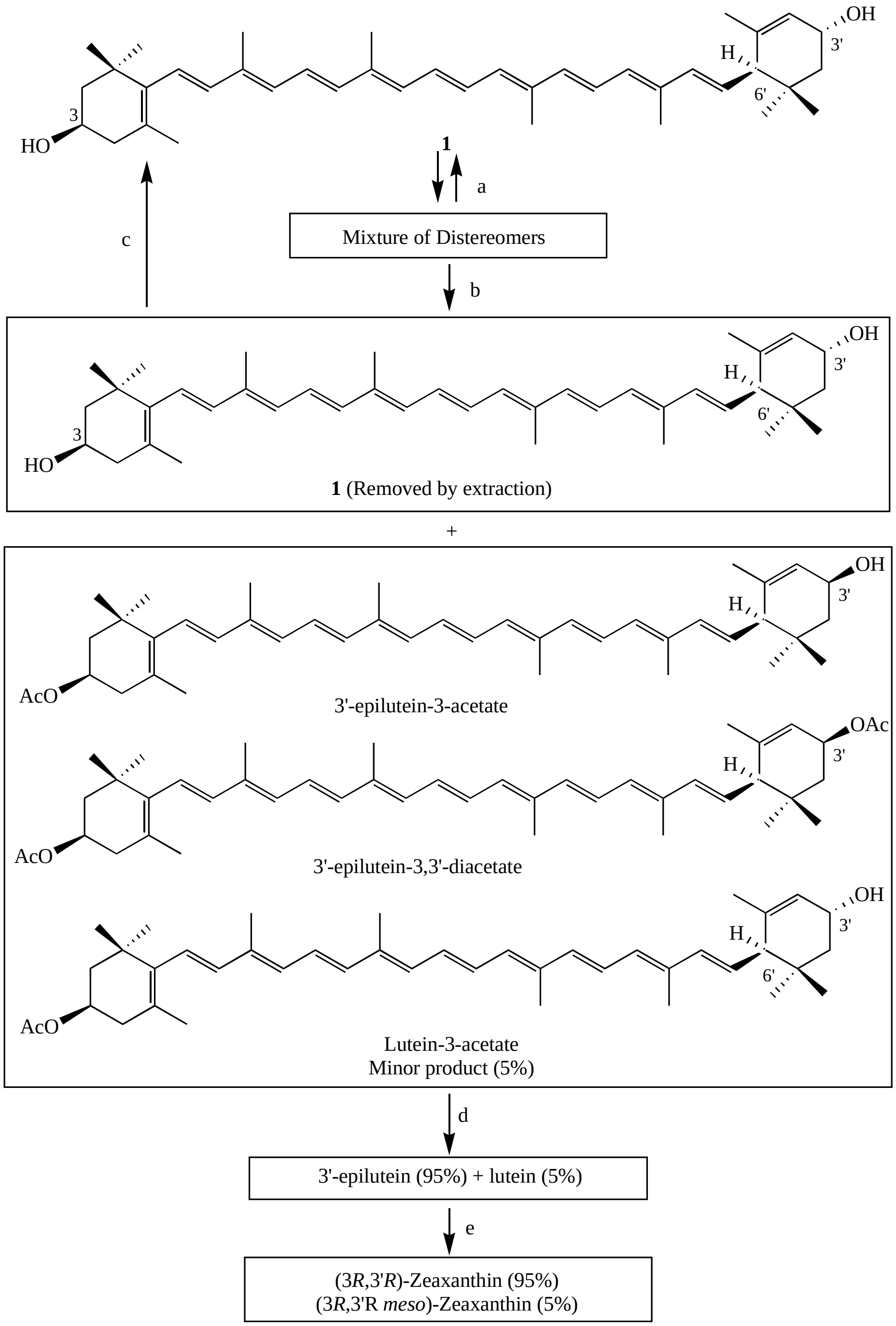
Anhydrolutein I (**8**) has also been synthesized by Buchecker *et al.* [17] from allylic reduction of lutein in good yield using a mixture of $AlCl_3$ and $LiAlH_4$ in a ratio 3:1 ($AlHCl_2$).

In 1995 Khachik *et al.* [18] published the partial synthesis of anhydrolutein I (**8**), II (**9**) and III (**7**) from the reaction of lutein with 2% sulphuric acid in acetone. These mild reaction conditions resulted in higher than 90% yields. The structures of the synthetic anhydroluteins (**7**-**9**), including their absolute configuration at C(3) and C(6') unambiguously were established by ^{1}H NMR and in part by ^{13}C NMR spectroscopies and circular dichroism. The prepared compounds were used as authentic samples for the exact identification of anhydroluteins in the extract of human plasma [18] and in human milk [18, 19].

2.2. Epimerization Reaction of Lutein

Khachik [20] described an efficient conversion of technical grade lutein to zeaxanthin (**10**) via 3'-epilutein (Scheme **1**). Acid-catalyzed epimerization of lutein (**1**) yielded an equimolar mixture of the diastereomers lutein and 3'-epilutein (**2**). The mixture was separated using enzyme-mediated acylation with lipase AK from *Pseudomonas fluorescens* that preferentially esterifies 3'-epilutein (**2**). After separation and alkaline hydrolysis 3'-epilutein was obtained in 90% diastereomeric excess (de). Compound 3'-epilutein (**2**) was also separated from lutein (**1**) in 56-88% de by solvent extraction and low-temperature crystallization, Soxhlet extraction or supercritical fluid extraction. Base-catalyzed isomerization of 3'-epilutein (**2**) gave zeaxanthin (**10**, Fig. **2**) in excellent yield, providing a convenient alternative to the total synthesis of this important dietary carotenoid.

In 2004 Molnár *et al.* [21] published an acid catalyzed epimerization reaction of lutein in THF/H_2O 1:1 mixture in the presence of aqueous HCl. This study established the same (3*R*,3'*S*,6'*R*)-configuration of epilutein samples of different origin: a) isolated from the extract of the flowers of *Caltha palustris*, b) prepared from 3'-oxolutein (**11**, Fig. **2**) by $NaBH_4$-reduction, and c) obtained from lutein (**1**) by acid-catalyzed epimerization.



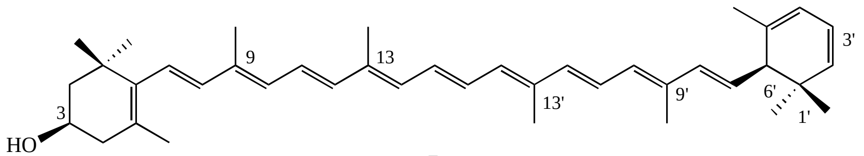
Scheme 1. Overall Sequence for Synthesis of 3'epilutein (**2**) and zeaxanthin (**10**) from lutein (**1**) [20]. (**a**) Acid-catalyzed epimerization, $H^+/THF/H_2O$, 25 °C; (**b**) enzyme mediated acylation with lipase AK or PS, vinyl acetate, pentane, reflux at 36 °C for 48-72 h; (**c**) extraction at 0 °C with a hydrocarbon solvent to remove carotenoid esters; (**d**) KOH/MeOH/THF, 25 °C; (**e**) base-catalyzed isomerisation, KOH/phase transfer catalyst, hexane, 64 °C, 5h.

2.3. Oxidation Reaction of Lutein

Chemical oxidation of the 3'-hydroxy group of lutein with MnO_2 or NiO_2 produced ((3*R*,6'*R*)-3-hydroxy-β,ε-carotene-3'-one) (**11**) in good yields [9, 22, 23] as the Oppenauer oxidation of lutein [24] did. 3'-Oxo-lutein (**11)** was isolated first from *Phylosamia cynthia* L. [24, 25] and has also been reported to be present in a number of other sources, e.g. eggs of flying fish (*Prognichthys agoo*), dolphin fish (*Coryphaena hippurus*), eggs of carps (*Cyprinus carpio* and *Cyprinus gibelio langsdorfi*), oily gudgeon (*Sarocheilichthys variegatus*) [12, 26] and two moth species [27]. Together with 3'-epilutein (**2**), 3'-oxolutein (**11**) has been identified in the extracts from marigold (*Tagetes erecta* L.) flowers [28] and has been detected in chicken egg yolk and in flowers of Marsh marigold (*Caltha palustris* L.) [29].

2.4. Partial Synthesis of (3*R*,6'*R*)-α-Cryptoxanthin and (3*R*)-*β*-Cryptoxanthin from (3*R*,3'*R*,6'*R*)-Lutein

Several processes have been developed by Khachik and his coworkers [30] for the transformation of lutein to α- and β-cryptoxanthin (**12, 13**, Fig. **3**) and minor quantities of (3*R*,5'*RS*,6'*R*)-3',4'-didehydro-5',6'-dihydro-*β,β*-caroten-3-ol (**14**) (a regioisomer of α-cryptoxanthin).



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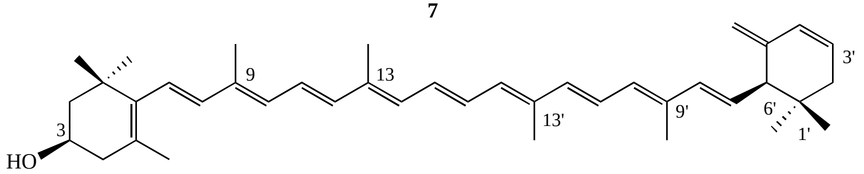

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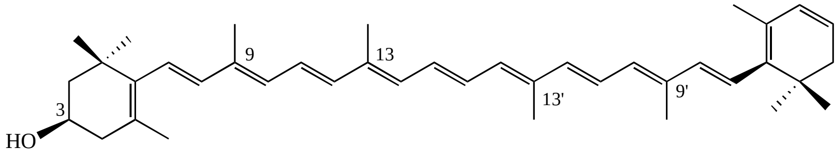

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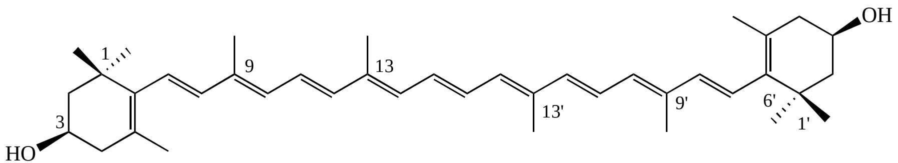

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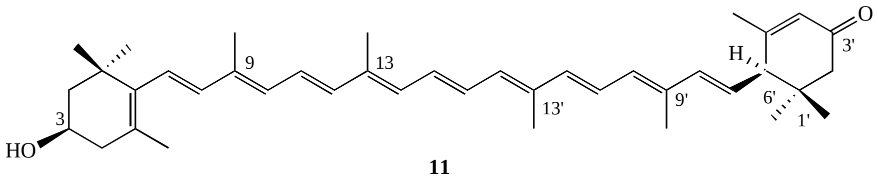

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Fig. (2).

With one of these methods lutein (**1**) was deoxygenated clearly to α-cryptoxanthin (**12**) in the presence of trifluoroacetic acid (TFA) and $Me_3N{\cdot}BH_3$ in CH_2Cl_2 at ambient temperature with nearly 90% yield. Reaction of lutein with a Lewis acid ($AlCl_3$, $ZnBr_2$, ZnI_2) and a hydride donor ($Me_3N{\cdot}BH_3$, $Na[BH_3(OCOCF_3)]$, $NaCNBH_3$) in solvents such as CH_2Cl_2, THF, and TBME produced similar results.

In a two-step process high-temperature acid-catalyzed dehydration of lutein (propanol/water/acid, 90 °C) gave a mixture of anhydroluteins I, II, and III (**7**-**9**) in 86% yield. In the second step these dehydration products underwent ionic hydrogenation with TFA/$Me_3N{\cdot}BH_3$ in CH_2Cl_2 to afford a mixture of α-cryptoxanthin and β-cryptoxanthin in ~80% yield that contained only 1% of (3*R*,5'*RS*,6'*R*)-3',4'-didehydro-5',6'-dihydro-*β*,*β*-caroten-3-ol (**14**).

2.5. Epoxidation of Lutein

All natural xanthophyll 5,6-epoxides hitherto elucidated exhibit a trans-relationship between the 3-OH and the epoxide group [31]. 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 (**15**, Fig. **3**), while the major product (~95%) had the (3*S*,5*S*,6*R*)-configuration (*syn*-lutein-epoxide, **16**) [32]. The exact characterization of *Z*-isomers of lutein 5,6-epoxide was published in 1988 [33].

2.6. Geometrical (*E*-*Z*) Isomerization of Lutein and 3'-epilutein

It has usually been considered that most carotenoids occur naturally as all-*E* isomers. The occurrence of some natural *Z* isomers is also demonstrated, however. Khachik *et al.* isolated (13*Z*,13'*Z*)-lutein from kale and marigold flowers [28]. All carotenoid isomers undergo reversible geometrical isomerization in solution. The main mono-*Z*-isomers (9*Z*, 9'Z, 13*Z* or 13'*Z*) and a di-*Z*-isomer (9*Z*,9'*Z*) both of lutein and 3'-epilutein were prepared by the thermal and the I_2-catalyzed photoisomerization of their solutions [34-36].

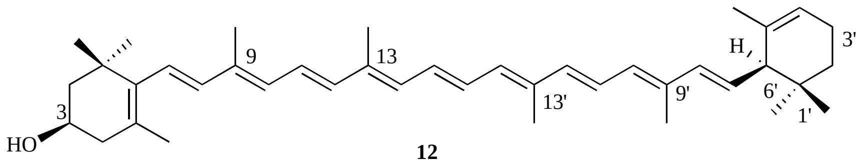
3. *IN VIVO* TRANSFORMATIONS OF LUTEIN

3.1. Lutein Derivatives in Foods

Carotenoids are one of the most abundant classes of phytochemicals found in nature. In foods, mainly in fruits and vegetables, more than 40 kinds of carotenoids are present. Among commonly consumed green vegetables lutein has the highest concentration in kale, parsley, spinach, sorrel and collards.

Lutein (**1**) was observed to convert to 3'-epilutein (**2**) and anhydrolutein I (**8**) in acidic media [18], but it had not been described yet during the food processing. From cooked sorrel (*Rumex rugosus* C.), Deli *et al.* isolated the newly formed 3'-epilutein (**2**) in crystalline form, and fully characterized by spectroscopic methods [37]. Since sorrel contains oxalic acid in larger amount, the epimerization reaction may take place during cooking when heat liberates the carotenoids and acid from the cells.

This recognition led Deli and his co-workers to investigate other vegetables and fruits which contain floral acids or which are stored in acidic conditions [38]. The epimerization and dehydration of lutein were also observed in cooked, baked and pickled green



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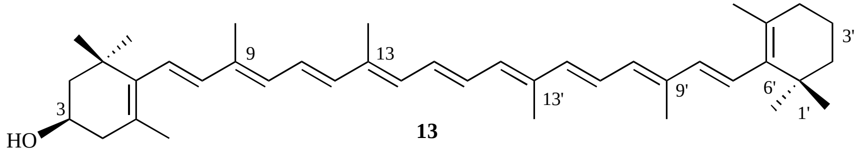

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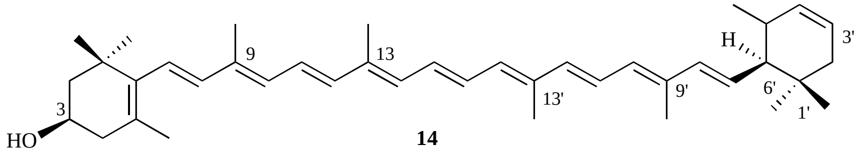

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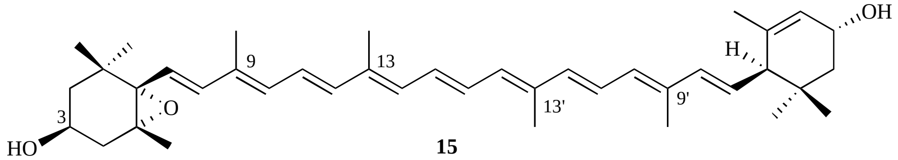

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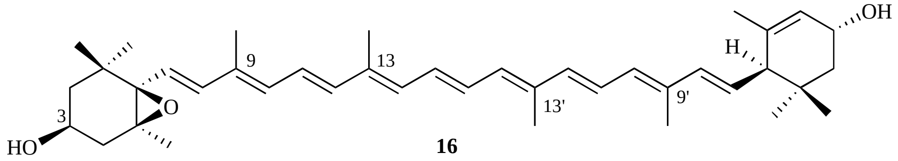

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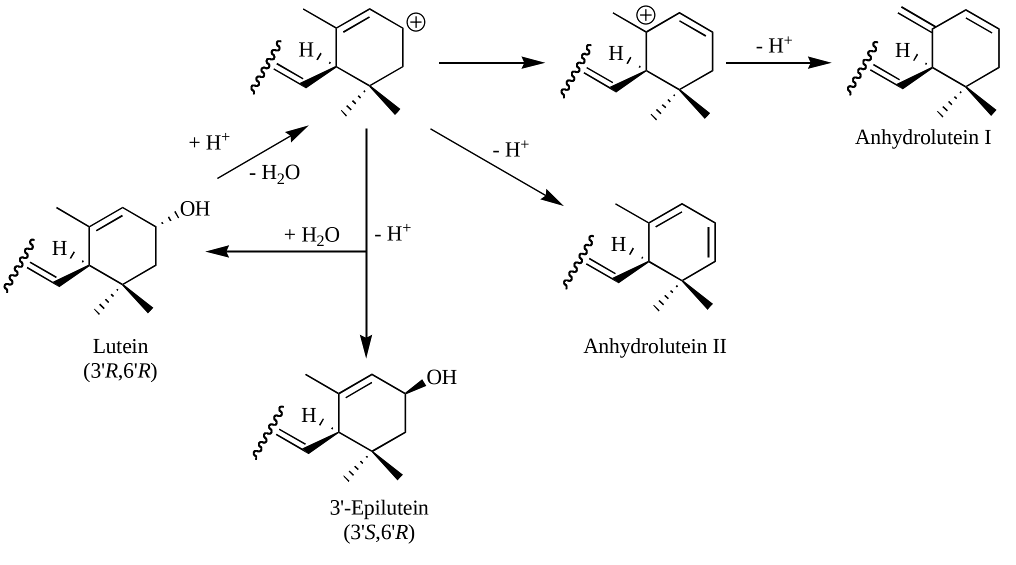
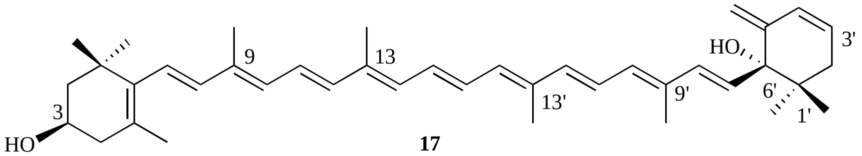
Fig. (3).



Scheme 2. Proposed transformations of lutein in foods [40].

paprika and in the extract of pickled dill. Similarly, the formation of 3'-epilutein and anhydrolutein I was detected in some bottled fruits (plum, blackberries) which do not contain these carotenoids in fresh state. At that time, due to insufficient amounts of samples for NMR analyses, the identification of anhydrolutein I (**8**) and II (**7**) was based solely on UV/VIS and mass-spectroscopic data [39]. One year later the full structure elucidation of anhydroluteins I (**8**) and II (**7**) isolated from the extract of cooked sorrel was reported [40]. The characterization of main *Z*-isomers of 3'-epilutein (**2**) and anhydrolutein I (**8**), which always formed during food processing, were also published [35, 41].

The water and acid contents of foods play an important role in the catalysis of the epimerization and/or dehydration reactions of different carotenoids [20]. The formation of an intermediate carbenium ion at C(3') is proposed during the epimerization and dehydration of lutein (**1**) (Scheme **2**). Reaction of this intermediate with a hydroxide ion can result epimers, both lutein (**1**) and 3'-epilutein (**2**) are formed [40]. Loss of a proton at C(2') results in the formation of anhydrolutein II (**7**). The resonance stabilized carbenium ion intermediate can undergo loss of a proton from C(18') resulting in the γ-end group of anhydrolutein I (**8**).



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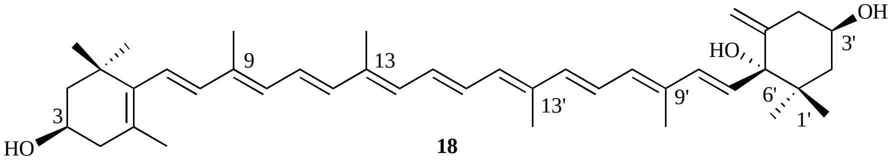

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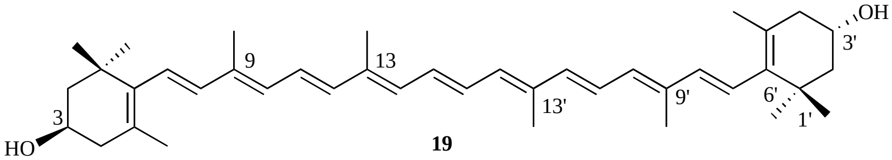

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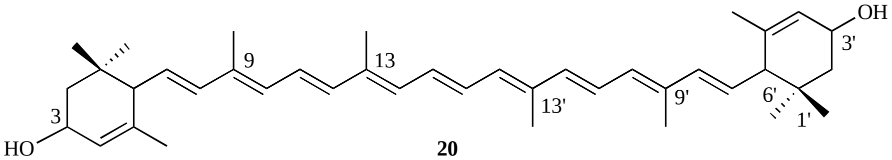

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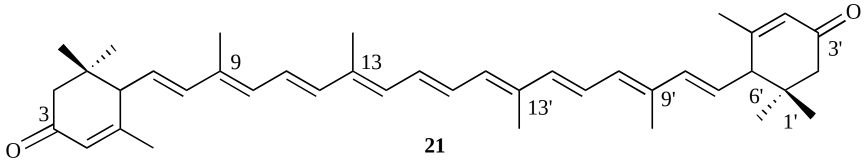

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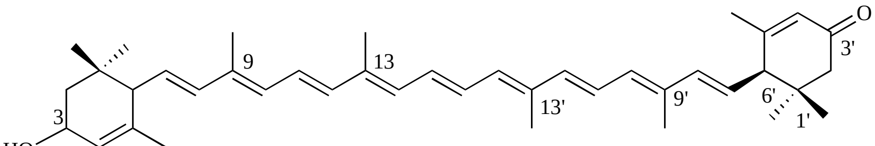

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Fig. (4).

3’-Epilutein (**2**) and the anhydroluteins **7** and **8** are rarely found in nature. Khachik reported the presence of anhydrolutein II (**8**) in a variety of squash [42, 43] and some anhydrolutein I was found in tomato purée [44]. The 6’-hydroxy-derivative of anhydrolutein I, namely nigroxanthin (**17**, Fig. **4**) [45] and the 3’,6’-dihydroxy-derivative of anhydrolutein I, i.e. prenigroxanthin (**18**) [46] were isolated from red paprika (*Capsicum annu*um) and characterized. Despite the limited dietary sources, Khachik and his co-workers [47] routinely detected anhydrolutein I (**8**) and II (**8**) by HPLC in the plasma extracts from subjects living in the United States. One explanation may be that the ingested dietary lutein, abundant in most fruits and vegetables, may serve as a precursor of anhydroluteins I and II in human organisms. Khachik *et al.* [47] suggested that anhydroluteins are formed in the human gastrointestinal tract by the acid-catalyzed dehydration of lutein in the stomach, similarly to the reaction pathway described earlier.

Our results demonstrated that some processed vegetables and fruits may serve as sources of 3’-epilutein (**2**) and anhydroluteins (**7** and **8**). Therefore, it is quite likely that the presence of lutein derivatives in human plasma may be due to both the absorption of 3’-epilutein and anhydroluteins, as well as metabolic conversion of dietary lutein (**1**) [40]. Studies on the biological activities and roles of 3’-epilutein (**2**) and anhydroluteins I -III (**7** - **9**) have not been published yet, which highlights the necessity for further investigations of the dietary intake and the biological role of lutein derivatives.

3.2. Lutein Derivatives in Humans

Carotenoids are normal constituents of the blood and tissues of humans, rumirants, birds, fish, and some crustaceans, but not of most rodents. In well-nourished humans, carotenoids are present primarily in the adipose tissue (80-85%), liver (8-12%), and muscle (2-3%), and smaller amounts in other tissues (3 %). The concentration of carotenoids, in contrast, is the highest in the corpus luteum (ca. 60 μg/g) and the adrenal gland (ca. 20 μg/g), they have lower concentrations in the adipose tissue and liver (ca. 10 μg/g); elsewhere, concentrations are even lower (0.5-3 μg/g). The total body pool of carotenoids (100-150 mg) in the serum of a well-nourished person usually contains ~1% and normally shows a concentration of 0.4-1.5 μg/ml (0.8-8 μmol/l). The total amount and concentrations of various carotenoids in the blood and tissues of an individual are highly dependent on the average daily intake of these pigments.

Lutein (**1**), 3-epilutein (**2**), 3’-oxolutein (**11**), anhydrolutein I (**8**), anhydrolutein II (**7**), zeaxanthin (**10**), and a number of other carotenoids and their oxidation products have been identified in the extracts of human plasma [17, 46, 47]. Lutein (**1**), zeaxanthin (**10**), *meso*-zeaxanthin (**19**, Fig. **4**), several (*Z*)-isomers of **10** and **19**, 3’-oxolutein (**11**), and 3’-epilutein (**2**) were detected in human and monkey retina [49] and in the tissues of the human eye [50, 51].

3.2.1. Lutein in Blood Plasma

Khachik *et al.* [18] reported that the formation of anhydroluteins in the human gastrointestinal tract (GI) could result from acid catalyzed dehydration of lutein (**1**) in the acidic gastric juice similar to the reaction pathway described earlier. Although the dehydration of lutein (**1**) in an aqueous medium in a biochemical environment, e.g. human GI tract, would be expected to be somewhat different than in organic solvents, the qualitative and quantitative distribution of the products suggest that there are similarities between the two processes. The dehydration of lutein (**1**) in organic solvents yields anhydrolutein I (**8**) as the major product and anhydrolutein (II) (**7**) as a minor product, while the average plasma concentration of lutein is 180-530 nmol/l, that of anhydroluteins is only 40-90 nmol/l.

According to Khachik this suggests that if anhydroluteins are formed from dietary lutein in the human digestive tract, less than 20% (based on molar equivalence) of lutein (**1**) is converted to anhydroluteins. This conversion most probably occurs within the first 1-2 h following ingestion when dietary lutein is subjected to the stomach acids. Although it may be only coincidental, these results are remarkably similar to the data obtained in the kinetic studies of acid catalyzed dehydration of lutein in organic solvents under various conditions. The kinetics experiments conducted by monitoring the quantitative and qualitative formation of the synthetic products by HPLC at various intervals have also indicated that after 1-2 h only about 15-20% of anhydroluteins were formed and 80-85% of lutein (**1**) remained unreacted [18].

In certain fruits and vegetables lutein is usually esterified with fatty acids such as palmitic and myristic acids. Therefore, the dietary lutein bispalmitate or lutein bismyristate may also lose palmitic or myristic acid in the acidic stomach to form the corresponding fatty acid esters of anhydroluteins. Dietary carotenol fatty acid esters, in general, have not been detected in human plasma. However, upon ingestion this class of carotenoids are believed to undergo hydrolysis in the presence of pancreatic secretions in the small intestine to cleave off their fatty acid moiety and to regenerate their parent hydroxycarotenoids [48, 52]. Consequently, if the fatty acid esters of anhydroluteins are formed in the stomach, they would similarly be converted to anhydroluteins which can then be absorbed and utilized by the body. In acidic organic solvents under similar conditions described earlier [18] the loss of long-chain fatty acids (palmitic or myristic acid) from lutein diesters proceeds much more readily than the loss of acetic acid from lutein diacetate. Therefore, it is quite likely that the presence of anhydroluteins in human plasma may be due to metabolic conversion of both the dietary lutein and lutein bisfatty acid esters [18].

3.2.2. Lutein and Eye Health

Lutein is known to many as “the eye-protective nutrient”. This reputation is borne from a comprehensive collection of research including studies demonstrating specific tissue deposition, observational studies showing an inverse relationship between lutein intake and eye disease and intervention studies showing that lutein supplementation results in increased lutein levels in the eye and even improved visual function in patients suffering from certain eye diseases.

In 1993 Bone *et al.* [53] elegantly established the stereochemistry of the human macular pigment and identified it as lutein [(3*R*,3'*R*,6'*R*)-β,ε-carotene-3,3'-diol] (**1**), zeaxanthin [(3*R*,3'*R*)-β,β-carotene-3,3'-diol] (**10**), and *meso*-zeaxanthin [(3*R*,3'*S*)-β,β-carotene-3,3'-diol] (**19**). In a case-control study published in 1994 high consumption of fruits and vegetables, rich specifically in lutein (**1**) and zeaxanthin (**10**), was correlated with a lower risk for age-related macular degeneration (AMD) [54].

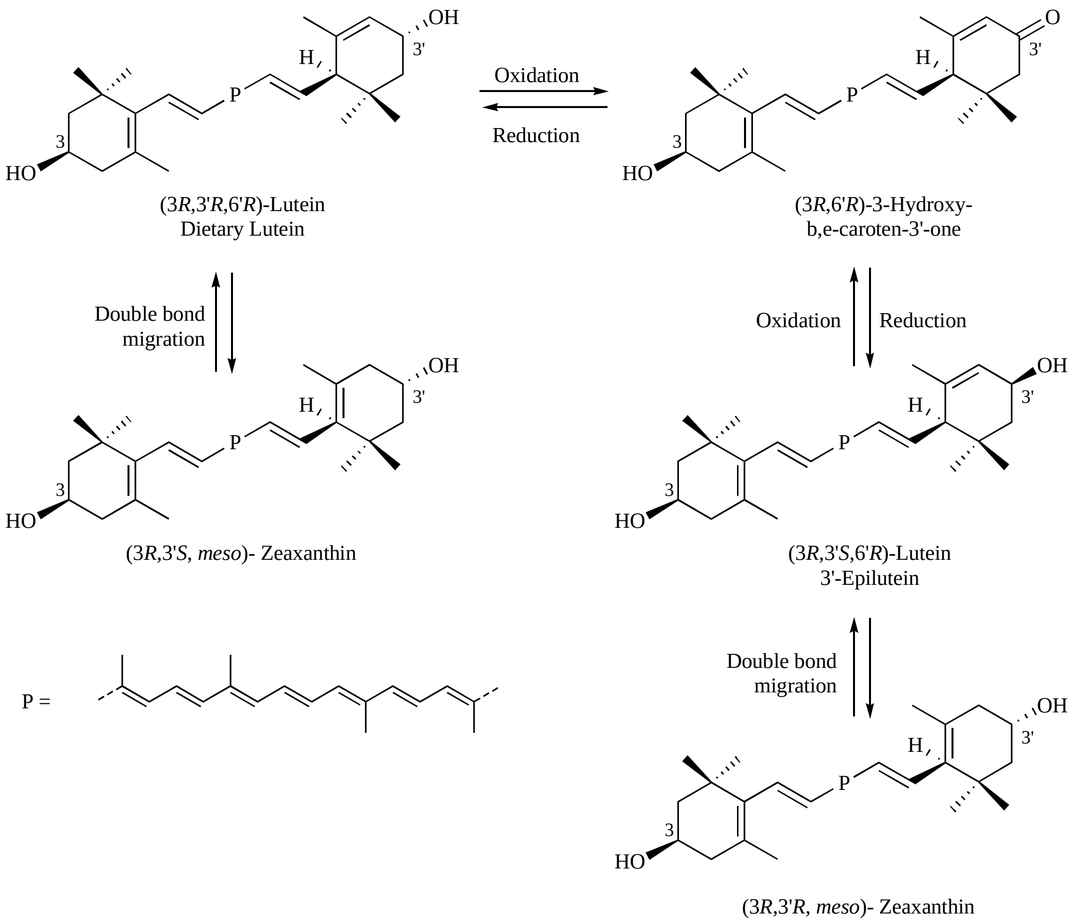
Besides lutein and zeaxanthin, several oxidation products of these compounds were detected in the extracts from human retina [49]. A major carotenoid resulting from direct oxidation of lutein (**1**) was identified as 3’-oxo-lutein (**11**). Minor carotenoids were identified as: 3'-epilutein (**2**), ε,ε-carotene-3,3'-diol (lactucaxanthin) (**20**), ε,ε-carotene-3,3'-dione (**21**) and 3-hydroxy-ε,ε-caroten-3’-one (**22**). Several geometric isomers of lutein (**1**) and zeaxanthin (**10**) were also detected at low concentrations. These were as follows: (9*Z*)-lutein, (9'*Z*)-lutein, (13*Z*)-lutein, (13'*Z*)-lutein, (9*Z*)-zeaxanthin, and (13*Z*)-zeaxanthin.

Among all the carotenoids identified in human retina only (all-*E*,3*R*,3'*R*,6'*R*)-lutein (**1**), (all-*E*,3*R*,3'*R*)-zeaxanthin (**10**), (all-*E*)-lactucaxanthin (**20**), and their (*Z*)-geometric isomers are of dietary origin. Although (3*R*,3'*R*,6'*R*)-lutein (**1**) is the most abundant carotenoid in all green and some yellow fruits and vegetables, the dietaiy sources of (3*R*,3'*R*)-zeaxanthin (**10**) are limited to corn, peaches, certain varieties of squash and citrus fruits [55]. These carotenoid oxidation products are not of dietary origin and have previously been identified in human plasma and breastmilk by Khachik *et al.* [19, 48]. It is not known if these carotenoid oxidation products are transported to and accumulated in retina *via* the circulatory system or whether photo-induced metabolic oxidation of lutein (**1**) and zeaxanthin (**10**) may be responsible for their presence. It is important to note that all of these oxidation products are present in both human plasma and retinas at very low concentrations. However, only the direct oxidation product of lutein (**1**), the 3'-oxo-lutein (**11**) is found in the retina in relatively high concentrations compared with other oxidative metabolites of carotenoids. Currently this finding provides the only evidence for a possible *in vivo* metabolic oxidation of lutein (**1**) in human retina. The protection of retina from short-wavelength visible light by lutein (**1**) and zeaxanthin (**10**) is based on two assumptions. First, the oxidation products of lutein and zeaxanthin are formed *in vivo* in the retina. Second, these oxidative metabolites are formed by the action of blue light. Based on several supplementation studies with purified lutein (**1**) and zeaxanthin (**10**) involving human subjects Khachik proposed metabolic pathways for conversion of these dietary carotenoids to their oxidation products [49, 52].

According to these metabolic transformations, (3*R*,3'*S*,meso)-zeaxanthin (**19**), (3*R*,3'*R*,6'*R*)-lutein (**1**), and (3*R*,3'*R*)-zeaxanthin (**10**) may be inter-converted through a series of oxidation-reduction and double bond isomerization reactions, as shown in Scheme **3**. The driving force for the direct oxidation of lutein to 3’-oxo-lutein (**11**) is the activation of the hydroxyl group at C-3' by the neighboring allylic double bond. However, the (3*R*,3'*R*)-zeaxanthin (**10**) is not directly oxidized because of the nonallylic nature of the hydroxyl groups at C-3 and C-3' and may therefore undergo double bond isomerization to yield 3'-epilutein (**2**) before oxidation. As a result, 3’-oxo-lutein (**11**) can also be formed from allylic oxidation of 3'-epilutein (**2**). To establish interconversion between carotenoids in these metabolic transformations, once 3-hydroxy-β,ε-caroten-3'-one (**11**) is formed it may undergo reduction reactions with epimerization at C-3' to yield lutein (**1**) and/or 3'-epilutein (**2**). In another double bond isomerization reaction dietary lutein (**1**) may be transformed into (3*R*,3'*S*,meso)-zeaxanthin (**19**). Therefore, the fact that Bone *et al.* [53] have demonstrated that almost equal amounts of (3*R*,3'*R*)-zeaxanthin (**10**) and (3*R*,3'*S*,meso)-zeaxanthin (**19**) are present in the human macula indicates that the latter compound may be formed from double bond isomerization of dietary (3*R*,3'*R*,6'*R*)-lutein (**1**) as shown in Scheme **3**. The proposed oxidative-reductive pathways for lutein and zeaxanthin in human retina, may therefore play an important role in prevention of age-related macular degeneration and cataracts.

4. CONCLUSIONS

In this review it was demonstrated that lutein and its derivatives play an important role in our everyday diet. After the extensive chemical investigations of lutein and its derivatives, their biological and medicinal importance induced a lot of studies in the fields of food chemistry, biochemistry and medicine in the last 10-15 years. Now we have quiet a good perspective about the mechanisms of the *in vitro* and *in vivo* transformations of lutein but still there are some



Scheme 3. Proposed metabolic transformations of dietary (3*R*,3’*R*,6’*R*)-lutein and (3*R*,3’*R*)-zeaxanthin in human retina [49].

unclarified facts mainly in medicinal applications which need further research in this area.

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