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Partial synthesis of serum carotenoids and their metabolites*

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Human serum and tissues contain in excess of 12 dietary carotenoids and several metabolites that originate from consumption of fruits and vegetables. Among these are hydroxycarotenoids: (*3R,3′R,6′R*)-lutein (1), (*3R,3′R*)-zeaxanthin (2), (*3R,6′R*)-α-cryptoxanthin (3), and (*3R*)-β-cryptoxanthin (4). In addition, several dehydration products of 1 have also been identified in human serum, these are: (*3R,6′R*)-3-hydroxy-3′,4′-didehydro-β,γ-carotene (5), (*3R,6′R*)-3-hydroxy-2′,3′-didehydro-β,ε-carotene (6), and (*3R*)-3-hydroxy-3′,4′-didehydro-β,β-carotene (7). Several metabolites of 1 and/or 2, namely, (*3R,3′S,6′R*)-lutein (3′-epilutein, 8) and (*3R,3′S;meso*)-zeaxanthin (9) have also been characterized in human serum and ocular tissues. Semi-synthetic processes have been developed that separately transform commercially available 1 into 4 *via* 7 as well as 1 into 8. While 8 is converted into 2 by base-catalyzed isomerization, 7 is transformed into 2 and its (*3R,3′S;meso*)-stereoisomer (9) by regioselective hydroboration.

Keywords: dietary hydroxycarotenoids, carotenoid metabolites, α-cryptoxanthin, β-cryptoxanthin, anhydroluteins, ionic hydrogenation, allylic deoxygenation, hydroboration

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INTRODUCTION

To date, 40-50 carotenoids have been identified in the extracts from commonly consumed foods; these can be classified as carotenoid epoxides, hydroxycarotenoids, hydrocarbon carotenoids, and carotenoid acyl esters (Khachik *et al.,* 1991). Among these, hydroxycarotenoids and hydrocarbon carotenoids are absorbed by humans and are found in plasma, breast milk, and various organs and tissues whereas carotenoid epoxides have not been detected in humans (Khachik *et al.,* 1992; 1997a; 2006). The major hydroxycarotenoids absorbed by humans are: (*3R,3′R,6′R*)-lutein (**1**), (*3R,3′R*)-zeaxanthin (**2**), (*3R,6′R*)-α-cryptoxanthin (**3**), and (*3R*)-β-cryptoxanthin (**4**) and their *E*/*Z*-stereoisomers (Fig. 1). In addition, several hydroxycarotenoids that result from metabolic transformation of **1** have also been identified in human plasma. Among are: (*3R,6′R*)-3-hydroxy-3′,4′-didehydro-β,γ-carotene (**5**) (*3R,6′R*)-3-hydroxy-2′,3′-didehydro-β,ε-carotene (**6**), and (*3R*)-3-hydroxy-3′,4′-didehydro-β,β-carotene (**7**) that are presumably formed by non-enzymatic acid-catalyzed dehydration of **1** (Khachik *et al.,* 1995). Hydroxycarotenoids **1** and **2** appear to undergo extensive metabolism to several ketocarotenoids by a series of oxidation-reduction and double bond isomerization reactions. For example, (*3R,3′S,6′R*)-lutein (3′-epilutein, **8**) and (*3R,3′S;meso*)-zeaxanthin (**9**) are among the metabolites of **1** and/or **2** in human serum and ocular tissues that are formed by such reactions (Bone *et al.,* 1993; Bernstein *et al.,* 1997; Khachik *et al.,* 1997b; Khachik *et al.,* 2002). The structures of these carotenoid metabolites are shown in Fig. 2.

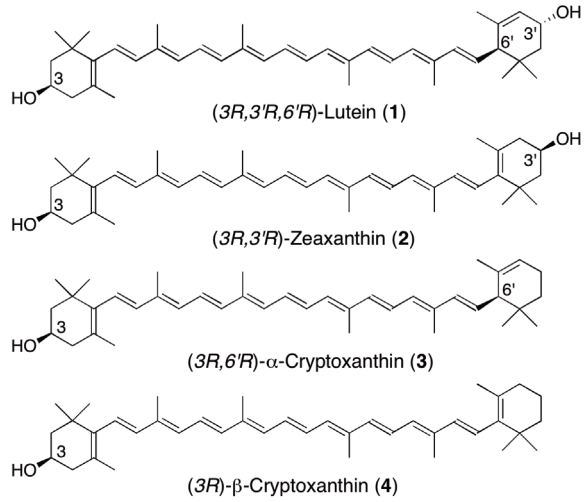

Figure 1. Structures of dietary hydroxycarotenoids found in human plasma, breast milk and ocular tissues.

Epidemiological and experimental evidence to date suggest hydroxycarotenoids may protect against chronic diseases such as cancer (Van Poppel, 1993), cardiovascular disease (Morris *et al.,* 1994) and age-related macular degeneration (AMD) (Seddon *et al.,* 1994). Therefore, supplementation with these carotenoids in individuals with a low dietary intake of fruits and vegetables is essential. The lack of commercial availability of some of these non-vitamin A active dietary carotenoids has limited the investigation of their metabolism and their biological activity. With the exception of **1** and **2**, industrial production of hydroxycarotenoids **3–9** have not yet materialized. While the total synthesis of **1** and four of its stereoisomers has been reported (Khachik & Chang, 2009), the isolation of this carotenoid from marigold flowers (*Tagetes erecta*) on industrial scale has proven to be the most viable and inexpensive route to this carotenoid (Khachik, 1995; Ausich & Sanders, 1997). In addition, **1** with stereocenters at 3,3′,6′-positions serves as an excellent precursor in the partial synthesis of optically active hydroxycarotenoids with ε- and β-end groups. An example of this is (*6′R*)-α-carotene (**10**) that could be ac-

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Abbreviations: BH_3-THF, borane-tetrahydrofuran; CH_3I, methyl iodide; $NaBH_4$, sodium borohydride.

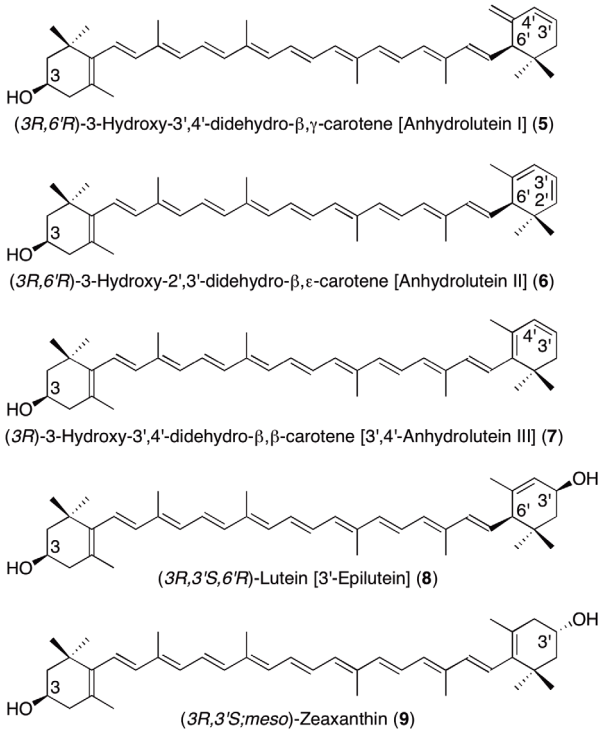


Figure 2. Structures of several metabolites of dietary (*3R,3′R,6′R*)-lutein (1) and (*3R,3′R*)-zeaxanthin (2); 5–9 have been identified in human plasma and breast milk while only 8 and 9 have only been identified in human ocular tissues.

cessible from two consecutive deoxygenation of **1** *via* **3** (Fig. 3).

Therefore, several relatively straightforward semisynthetic processes have been developed that transform **1** into **3–10** in excellent yields and high optical purities. These processes provide easy access and alternative routes to optically active carotenoids that are normally prepared by multi-step synthesis.

RESULTS AND DISCUSSION

To accomplish the synthesis of hydroxycarotenoids, (*3R,3′R,6′R*)-lutein (**1**) that is commercially available from saponified extracts of marigold flowers (*Tagetes erecta*) has been employed as the key starting material. The allylic deoxygenation of **1** at the 3-position under mild reaction conditions to **3** was accomplished by a number of reagents under mild conditions in excellent yields (Khachik *et al.,* 2007) (Scheme 1). In another approach, **1** was subjected to ionic hydrogenation with triethylsilane/trifluoroacetic acid (Et_3SiH/TFA) that is a known reagent for the reduction of multiple bonds and deoxygenation of single bonds such as C-OH (Kursanov *et al.,* 1974). This resulted in the formation of a mixture of **3** and **4** as well as a mixture of lutein dehydration products that were identified as (*3R,6′R*)-3-hydroxy-3′,4′-didehydro-β,γ-carotene [anhydrolutein I] (**5**), (*3R,6′R*)-3-hydroxy-2′,3′-didehydro-β,ε-carotene [anhydrolutein II] (**6**), and (*3R*)-3-hydroxy-3′,4′-didehydro-β,β-carotene [anhydrolutein III] (**7**) (Khachik *et al.,* 2007). Carotenoids **5**, **6**, and **7** are the dehydration products of dietary lutein that have been isolated and characterized in extracts from human plasma; these carotenoids are presumably formed in human digestive system in the presence of acids (Khachik *et al.,* 1995). It should be noted that **8** is also a precursor of vitamin A2. Following the course of this reaction, it was revealed that **1** was first dehydrated to **5**, **6**, and **7** and subsequently these carotenoids were in part converted to **3** and **4**. Low temperature acid-catalyzed dehydration of lutein has been shown to yield predominantly **5** as the

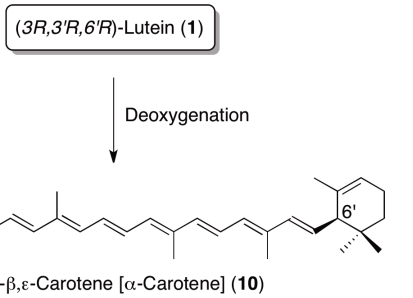
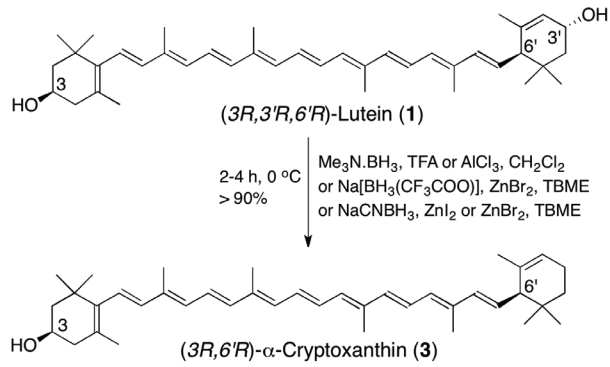
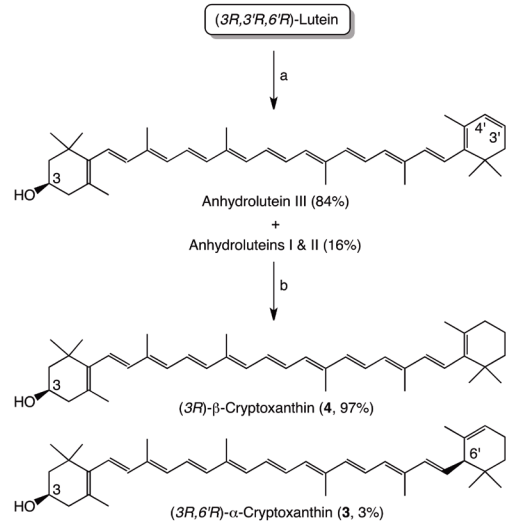


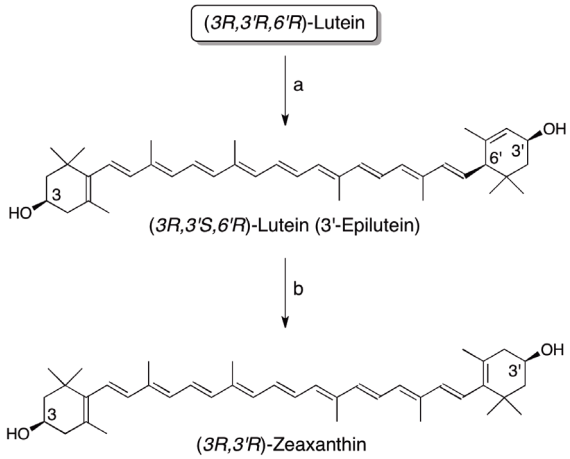
Figure 3. (*3R,3′R,6′R*)-lutein (1) as a possible precursor of (*6′R*)-α-carotene (10).



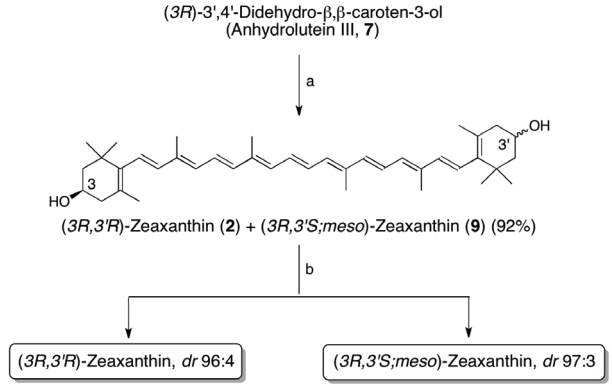
Scheme 1. Partial synthesis of (*3R,6′R*)-α-cryptoxanthin (3) from (*3R,3′R,6′R*)-lutein (1); TBME: *tert*-butyl methyl ether (Khachik *et al.,* 2007).



Scheme 2. Two-steps transformation of (*3R,3′R,6′R*)-lutein (1) to (*3R,6′R*)-α-cryptoxanthin (3) and (*3R*)-β-cryptoxanthin (4) *via* anhydroluteins 5, 6, and 7; step one: dehydration of 1, (a) HCl, H_2O, propanol (PrOH), 45–90°C; step two: regioselective ionic or catalytic hydrogenation of a mixture of 6–8, (b) trifluoroacetic acid (CF_3CO_2H), borane-trimethylamine ($Me_3N.BH_3$), dichloromethane (CH_2Cl_2); H_2/catalyst/ CH_2Cl_2 or ethyl acetate (EtOAc); two consecutive crystallization (CH_2Cl_2/EtOH) (Khachik *et al.,* 2007).



Scheme 3. Partial synthesis of (*3R,3′R*)-zeaxanthin (2) from (*3R,3′R,6′R*)-lutein (1) *via* 3′-epilutein (8); a) 1. HCl (5%), H_2O, tetrahydrofuran (THF), 2. low-temperature crystallization; 2. base-catalyzed isomerization, KOH (9 M), phase transfer catalyst, hexane (reflux) (Khachik *et al.,* 2003).



Scheme 4. Partial synthesis of (*3R,3′R*)-zeaxanthin (2) and (*3R,3′S;meso*)-zeaxanthin (9) from (*3R,3′R,6′R*)-lutein (1); a) 1. BH_3-THF, 2. NaOH (3N), H_2O_2 (30%), 3. crystallization; b) 1. lipase PS (*Pseudomonas cepacia*) or lipase AK (*Pseudomonas fluorescens*), vinyl acetate, THF, r.t., 2. chromatography (Khachik, 2009).

major product and **6** and **7** as the minor products. Because **5** and **6** appeared to have been converted to **3** by ionic hydrogenation, this approach resulted in the formation of a complex mixture of products in which **3** was the major product and **4** the minor product. Because the ionic hydrogenation of **7** was most likely responsible for the formation of **4**, the low yield of this carotenoid appeared to be due to the low yield of its precursor.

Therefore, in an attempt to transform **1** to **4** a two-step process was developed. In the first step, **1** was first dehydrated to a mixture of **5**, **6**, and **7** at high temperature to obtain the thermodynamically stable **7** as the major product. This was accomplished in a refluxing solution of **1** in propanol-water (PrOH-H_2O) at 90°C in the presence of catalytic amounts of hydrochloric (HCl) or sulfuric acid (H_2SO_4) in which anhydroluteins I (**5**) and anhydrolutein II (**6**) underwent isomerization to anhydrolutein III (**7**) within 12–18 h to yield a mixture of anhydroluteins in 85% yield in which the composition of the mixture was determined by HPLC as **7** (86%), **6** (9%), and **5** (5%). The ionic hydrogenation of the resulting mixture of anhydroluteins afforded a mixture of **4** (88%) and **3** (12%) in yields greater than 80%. Following two consecutive crystallizations, the ratio of **4** to **3** could be improved to 97:3 (Scheme 3) (Khachik *et al.,* 2007).

Another process has also been developed that converts **1** into (*3R,3′R*)-zeaxanthin (**2**) *via* (*3R,3′S,6′R*)-lutein (3′-epilutein, **8**) as shown in Scheme 3 (Khachik, 2003). This involved epimeriziation of **1** in dilute acidic solution followed by low-temperature crystallization that allowed the separation of **8** from unreacted **1**. The base-catalyzed isomerization of **8** with KOH (9 M) in refluxing hexane in the presence of phase transfer catalyst afforded **2** in high yield. This step was carried out similar to the base-catalyzed isomerization of 1 to (*3R,3′S;meso*)-zeaxanthin (**9**) that was first reported by Bernhard and Giger (1998).

Anhydrolutein III (**7**) has also been shown to serve as a useful precursor in the synthesis of (*3R,3′R*)-zeaxanthin (**2**) and its meso-isomer (**9**). Hydroboration of **7** with BH_3-THF prepared in situ from $NaNH_4/CH_3I$ afford a mixture of **2** and **9** that were separated by enzyme-mediated acylation with lipase PS (*Pseudomonas cepacia*) or lipase AK (*Pseudomonas fluorescens*) in high diastereomeric ratio (*dr*) as shown in scheme 4 (Khachik, 2009).

CONCLUSION

The semisynthetic approach to optically active hydroxycarotenoids and their metabolites provides an alternative to total synthesis and can minimize the difficulties associated with multistep synthesis and, in addition, affords a much higher yield of the desired product. Commercially available (*3R,3′R,6′R*)-lutein (**1**) has been shown to serve as a key starting material in partial synthesis of (*3R,6′R*)-α-cryptoxanthin (**3**) and (*3R*)-β-cryptoxanthin (**4**) in two convenient steps *via* anhydroluteins I – III (**5**–**7**) in high yield. In addition, **1** can also be directly converted to **3** in a single step in greater than 90% yield. In another process, **1** has been efficiently converted to (*3R,3′R*)-zeaxanthin (**2**) *via* 3′-epilutein (**8**) that is a metabolite of **1** and/or **2** identified in human serum, breast milk, and ocular tissues. In an alternative process **1** has been transformed into a mixture of **2** and (*3R,3′S;meso*)-zeaxanthin (**9**) that were separated by enzyme-mediated acylation. These methodologies provide an easy access to optically active hydroxycarotenoids that have been shown to exhibit biological activities and enables researchers to further study the bioavailability and efficacy of these carotenoids in the prevention of chronic diseases.

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