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Transformation of naturally occurring (3R,3′R,6′R)-lutein and its fatty acid esters to (3 R)-β-cryptoxanthin and (3R,6′R)-α-cryptoxanthin☆

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ABSTRACT

The objective of this study was to develop straightforward processes that could be applied to the large-scale production of β-cryptoxanthin in an attempt to facilitate investigation of its biological activity. An oleoresin obtained from crude extracts of marigold flowers (*Tagetes erecta*) with approximately 24% total lutein fatty acid ester content was directly used as starting material for partial synthesis of (*3 R*)-β-cryptoxanthin under mild reaction conditions at ambient temperature. Therefore, acid-catalyzed deoxygenation of lutein esters from marigold oleoresin followed by hydrogenation in the presence of catalytic amount of platinum (Pt) supported on alumina (5%) at ambient temperature gave a mixture of (*3 R*)-β-cryptoxanthin fatty acid esters (major) and (*3 R,6′R*)-α-cryptoxanthin fatty acid esters (minor). Saponification and *Z*-to-*E* isomerization of the product followed by crystallization gave a mixture of (*3 R*)-β-cryptoxanthin as the major product. Similarly, acid-catalyzed hydrogenation of unesterified (*3 R,3′R,6′R*)-lutein with Pt/alumina in ethyl acetate gave a mixture of (*3 R,6′R*)-α-cryptoxanthin acetate (minor) in a one-pot reaction. Alkaline hydrolysis and *Z*-to-*E* isomerization of the mixture followed by crystallization provided (*3 R*)-β-cryptoxanthin.

1. Introduction

In the past 50 years in excess of 600 carotenoids have been synthesized and/or isolated from natural products with contributions from many distinguished chemists [1]. This has allowed scientists in various disciplines to discover the important role of these pigments in nature and particularly as it relates to human health. More recently, the concept of green chemistry that avoids the creation of toxics and waste has limited the lengthy multi-step synthesis of carotenoids. This has led to the preference for isolation of carotenoids from natural products by employing food grade solvents and reagents. Because some carotenoids are rare in nature, their isolation from natural products is not economically viable. Therefore, the most practical approach to access these carotenoids is by structural modification of those that are abundant. This semisynthetic concept that is known as partial synthesis in carotenoid chemistry was first published in 1971 [2]. (*3 R,3′R,6′R*)-Lutein, (*3 R,3′R*)-zeaxathin, (*3 R,6′R*)-α-cryptoxanthin and (*3 R*)-β-cryptoxanthin are among the major hydroxycarotenoids in human plasma and ocular tissues with potentially important health promoting properties. This project was motivated due to several published studies that have indicated the health benefit of (*3 R*)-β-cryptoxanthin on bone growth and inhibition of bone resorption [3,4]. This provitamin A dietary carotenoid is also present in human plasma and breastmilk [5] as well as ocular tissues, prticularly in the human ciliary body and may protect this tissue against ocular conditions such as presbyopia and glaucoma [6]. Further, high plasma concentrations of (*3 R*)-β-cryptoxanthin and several other carotenoids in human subjects have been associated with a reduction in blood pressure [7]. The immunostimulatory effect of (*3 R*)-β-cryptoxanthin in mice and in rabbits in a series of *in vitro* and *in vivo* studies have suggested that this carotenoid may play an important role in promoting human health [8]. More importantly, a human study has concluded that carotenoid-rich dietary pattern that includes (*3 R*)-β-cryptoxanthin, may contribute to the preservation of cognitive functions during aging [9]. Therefore the commercial availability of this carotenoid allows investigators to evaluate its biological activities that can contribute to human health.

The first process for the conversion of lutein to a mixture of (*3 R*)-β-cryptoxanthin and (*3 R,6′R*)-α-cryptoxanthin, was reported by Khachik [10]. This process involved conversion of lutein (**1**) containing approximately 5–7% (*3 R,3′R*)-zeaxanthin (**2**) to a mixture of (*3 R*)-β-cryptoxanthin (**6**) and (*3 R,6′R*)-α-cryptoxanthin (**7**) *via* (*3 R,6′R*)-anhydrolutein I [(*3 R,6′R*)-3-hydroxy-3′,4′-didehydro-β,γ-carotene] (**3**), (*3 R,6′R*)-2′,3′-anhydrolutein II [(*3 R,6′R*)-3-hydroxy-2′,3′-didehydro-β,ε-carotenene] (**4**), and (*3 R*)-3′,4′-anhydrolutein III [(*3 R*)-3-hydroxy-3′,4′-didehydro-β,β-carotene] (**5**) in one synthetic step by allylic deoxygenation with a strong acid and a hydride ion donor. The

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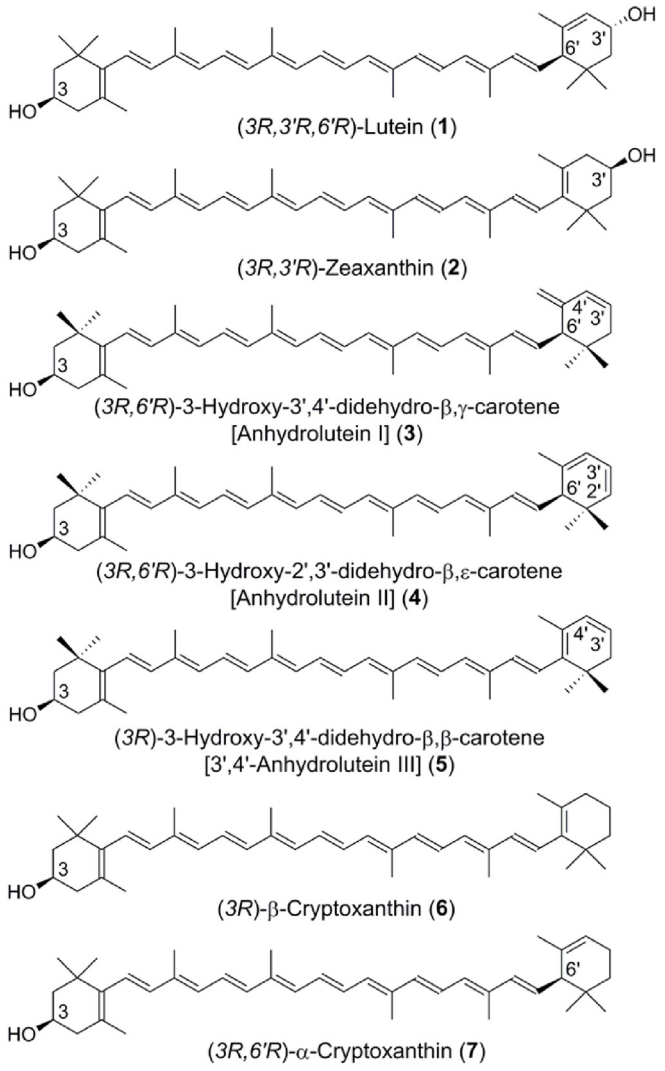

Fig. 1. The chemical structures of (*3 R,3′R,6′R*)-lutein (**1**), (*3 R,3′R*)-zeaxanthin (**2**), anhydroluteins I (**3**), II (**4**), and III (**5**), (*3 R*)-β-cryptoxanthin (**6**), and (*3 R,6′R*)-α-cryptoxanthin (**7**). The systematic names of anhydroluteins are shown below their structures.

chemical structures of these carotenoids are shown in Fig. 1.

Acid-catalyzed dehydration of lutein in a homogenous phase was carried out in a variety of solvents such as ethers (THF, *tert*-butyl methyl ether), chlorinated solvents (dichloromethane, chloroform, 1,2-dichloroethane), acetone, and toluene at ambient temperature. However, this led to the formation of considerable amount of *Z* (*cis*)-isomers of anhydroluteins. Further, under reaction conditions, anhydrolutein I (**3**) was the major product and anhydroluteins II (**4**) and III (**5**) were the minor products. Among these, **5** and **4** were precursors of (*3 R*)-β-cryptoxanthin (**6**) and (*3 R,6′R*)-α-cryptoxanthin (**7**), respectively while **3** could lead to the formation of both **6** and **7**. As a result, the ionic hydrogenation of these anhydroluteins led to a mixture of **6** and **7** in the ratio of 1.6:1 (Scheme 1) [10].

In a modified two-step process, the dehydration of **1** was significantly improved to increase the ratio of anhydrolutein **5** relative to **3** and **4** [11,12]. In the first step, lutein (**1**) was dehydrated in 1-propanol and aqueous acid at 78–88 °C to a mixture of **5** (82%), **4** (6%), and **3** (12%), nearly quantitatively (Scheme 2). In the second step, the mixture was subjected to ionic hydrogenation in dichloromethane to afford a mixture of **6** and **7** in the ratio of 3:1 [11,12]. The reduction step was further modified by eliminating the use of chlorinated solvents and reagents such as trifluoroacetic acid and hydride donors. This was accomplished by heterogeneous regioselective catalytic hydrogenation of anhydroluteins at 40 °C to afford a mixture of **6**:**7** = 5:1 as shown in Scheme 2 [13]. Although, this two-step process provided **6** in excellent yield and high purity, it was anticipated that this transformation could be modified to avoid high temperature and a costly starting material. This report describes a straightforward methodology for direct transformation of lutein esters from crude extracts of marigold flowers to (*3 R*)-β-cryptoxanthin (**6**) as the major product at ambient temperature employing green chemistry. This methodology has also been successfully applied to the preparation of **6** from commercially available purified lutein.

2. Results and discussion

Marigold flowers (*Tagetes erecta*) are the most abundant source of commercially available and purified (*3 R,3′R,6′R*)-lutein (**1**, 94%) that is accompanied by minor amounts of (*3 R,3′R*)-zeaxanthin (**2**, 6%). In marigolds these carotenoids are esterified with palmitic and myristic fatty acids. An oleoresin obtained from crude extracts of marigold flowers that contained approximately 24% total lutein and zeaxathin fatty acid esters was transformed to a mixture of (*3 R*)-β-cryptoxanthin (**6**, 98%) and (*3 R,6′R*)-α-cryptoxanthin (**7**, 2%) in three steps. The overall process for this transformation is shown in Scheme 3. In the first step, acid-catalyzed deoxygenation of lutein esters at the allylic position led to the formation of anhydrolutein acyl esters that underwent hydrogenation with Pt/alumina (5%) at ambient temperature to afford acyl esters of **6** and **7**. This was followed by saponification of the esters to afford their corresponding hydroxycarotenoids that in the same step were subjected to *Z*-to-*E* isomerization. In the final step, crystallization of the crude product lead to a mixture of **6** (98%) and **7** (2%). It should be noted that the isolated hydroxyl group in lutein esters remained unreactive throughout these reactions. Similarly, (*3 R,3′R)*-zeaxanthin esters with two isolated hydroxyl groups did not undergo deoxygenation and were removed from the purified reaction product by crystallization. The most interesting aspect of this process was the number of reactions that took place in one-pot to afford (anthin esters. In the second step of this transf)-β-cryptoxanthin acyl esters. In a sequential order, these reactions were: 1) acid-catalyzed hydrolysis of lutein esters at the allylic position, 2) acid-catalyzed dehydration of the resulting allylic hydroxyl group to anhydrolutein acyl esters, 3) acid-catalyzed isomerization of anhydrolutein acyl esters, 4) acid-catalyzed transesterification of anhydrolutein acyl esters in ethyl acetate (solvent), and 5) catalytic hydrogenation of anhydrolutein acyl esters to (anthin esters. In the second step of this transf)-β-cryptoxanthin esters and (*3 R,6′R*)-α-cryptoxanthin esters. In the second step of this transformation, (*3 R*)-β-cryptoxanthin acyl esters were saponified and isomerized to afford a crude mixture of *all-E*-**6** and **7** that in the third step were further purified by crystallization [14]. According to this process, the overall yield of **6** and **7** from lutein esters was 70%.

In a typical reaction, a solution of marigold oleoresin in ethyl acetate was treated with Pt/alumina (5%) and 50% H_2SO_4/H_2O (v/v) and the mixture was stirred at ambient temperature under an atmosphere of hydrogen overnight. The sequence of the reactions shown in Scheme 3 was established by closely monitoring the course of the one-pot reaction by HPLC that revealed the formation of the products of each step (see supporting information). In the initial step, lutein fatty acid esters were deoxygenated at the allylic position to yield anhydrolutein fatty acid esters while the non-allylic hydroxyl groups remained unchanged. This acid-catalyzed allylic deoxygenation was quite interesting because protonated esters are not particularly a very good leaving group in comparison with protonated alcohols. Therefore, the formation of anhydrolutein fatty acid esters under acidic conditions was attributed to the initial hydrolysis of the allylic acyl esters of lutein to their corresponding allylic alcohols followed by dehydration. On the other hand, it has been well established that acid catalyzed hydrolysis of an ester to an alcohol is a very slow reaction at ambient temperature