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Chemical synthesis project. A new yellow carotenoid*

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Abstract: Naturally occurring colorants have been used in food processing for centuries to give meals an appealing color. In the first half of the 20th century, the newly discovered brilliant azo dyes, amongst other artificial colorants (indol, triphenylmethane, and methine dyes), were used as pigments for food coloration. The toxicity and/or allergenic potential of some of these colorants were discovered much later. One of these pigments with a critical safety profile is the azo dye tartrazine, which exhibits a nicely fresh greenish-yellow color. The use of tartrazine is now banned in several countries and restricted in others due to its unfavorable safety profile.

With the aim of extending the color fan of nature-identical food colorants offered by Roche and therefore offering a less critical colorant to the food industry, a project was initiated at Roche. The goal was to find a safer, naturally occurring pigment with a color hue similar to tartrazine.

This paper discusses the process of how such a project is addressed in industry, as well as how promising candidates were selected from the wide variety of the naturally occurring carotenoids. The syntheses of some of these carotenoids will also be described.

INTRODUCTION

The color of the food we consume has a direct connection with how we are attracted to it. When consuming a certain type of food, we associate it with a distinctive color. We expect salmon to exhibit an appealing red-orange tone, whereas an appetizing banana should have a lush yellow color. If, for example, the color of the flesh of a salmon is gray-white, we would most likely be discouraged from eating it. Such a color simply does not reflect what we expect from a fresh and healthy salmon. Our experience has taught us that the color of our food is closely related to freshness and, therefore, healthiness or authenticity of the respective foodstuff. For a long time, food producers have used natural or artificial colorants to give their products a fresh and natural appearance.

In recent times, people have become more and more suspicious of the types of artificial ingredients that are added to our everyday diet. If critical consumers do not perceive a substantial benefit from an added ingredient (e.g., an enhanced stability by the addition of antioxidants), they might not buy this product. Instead, they might switch to an alternative, more nature-like product that does not contain the questioned ingredient. This consumer awareness is probably most pronounced in central and northern Europe.

Health authorities in some countries have also begun to question and even to ban the use of certain food additives. Some affected ingredients are also food colorants. Currently, there are still numerous pigments in use as food colorants that do not occur in nature (e.g., azo dyes like tartrazine, polymethine dyes, and other classes). Many consumer organizations have focused on food colorants,

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sometimes labeling them as unnecessary ingredients. It is very easy to find numerous Web sites where certain food colorants are cited or suspected as having possibly severe side effects upon digestion.

Roche offers a wide range of nature-identical pigments as food colorants in the red–orange and yellow–orange color palette. A yellow color with a fresh greenish tint, however, is still missing. This segment of the color palette is occupied only by artificial colorants that are not produced by Roche, such as the azo dye tartrazine (E 102, acid yellow 23, FD&C yellow 5, CI food yellow 6) or quinoline yellow (E 104, acid yellow 3, FD&C yellow 10, CI food yellow 13). These two colorants are examples of two heavily criticized artificial ingredients in human food.

To expand the Roche palette into this part of the color spectrum, a project has been initiated with the aim of finding a nature-identical carotenoid with the desired color hue. This carotenoid would then offer food producers a safe alternative to the two artificial colorants tartrazine and quinoline yellow.

Crystalline, nature-identical carotenoids produced by chemical synthesis are ideal colorants for food and feed applications. They offer the advantage of high purity and uniformity and are not suspected of any toxicological effects.

OUTLINE OF THE PROJECT

Prerequisites for a successful candidate

The project team has set up a set of prerequisites for a successful candidate. The most important ones are the following:

- **Natural occurrence in the human food chain.** The target compound has to occur widely in foods consumed by humans. This requirement should assure the customer's acceptance, which in the field of food ingredients plays a key role in the marketability of a product.
- **Ease of synthesis.** The synthesis of the target carotenoid should be simple to assure low production cost. The food coloration market is a very price-sensitive market.
- **Ease of formulation.** Since a target compound needs to be formulated, it has to be readily soluble in an organic solvent that is not water-soluble.
- **Stability of the color.** The color of the target compound has to be stable. While this may be obvious, it is an important issue as carotenoids are sensitive toward several conditions (e.g., heat, light, air, sometimes acidic or basic conditions). The formulation process can prevent this to some extent.
- **Correct color hue.** The most important point is that the target compound has to exhibit the desired yellow with a greenish hue.

The above-mentioned points are by nature strongly dependent on each other, therefore these points have to be addressed repeatedly throughout the process.

Organization of a project team

To tackle such a task in industry, a project team has to be organized. In this team, several functions have to be represented:

- **Chemistry:** to identify and synthesize possible target structures
- **Formulation/application:** to render the nonwater-soluble carotenoids in a water dispersible form and also to shift the color of this dispersion in the desired direction and to test this formulation in a test application (drink, yogurt, candies, etc.)
- **Chemistry/formulation:** to prove the technical feasibility
- **Biology/toxicology:** to prove the safety of the target compound in human consumption
- **Biology/toxicology/registration:** to check and set the basis for the registration process of the target compound as a new food ingredient in different markets worldwide
- **Marketing:** to make an estimation of the size of a possible market and calculate a business case

SELECTION PROCESS

The whole selection process is represented in Fig. 1 as a multistage funnel, where the filter size is reduced from one stage to the next. Translated to our project, the number of target carotenoids is systematically restricted down to one ultimate compound.

The prerequisites for a successful candidate set the guidelines for the class of compounds to be searched as a potential target.

"Natural occurrence in the human food chain" is an important restriction. We, therefore, started to search for carotenoids listed in *Key to Carotenoids* [1] and the supplementary list of newly discovered carotenoids listed in *Carotenoids 1A* [2]. In these books, only naturally occurring carotenoids are listed. However, the large majority of them do not occur in the human food chain in significant amounts. To separate them from the carotenoids isolated from organisms that do not belong to the human food chain (e.g., insects, algae, nonedible fruits and mushrooms, and other sources) requires a lot of experience and know-how from several persons familiar with the field of carotenoids and an intensive literature search. At this stage of the process, we selected (also taking into consideration that the structural motive of the β,β-carotene polyene backbone exhibited an undesired strong orange color) some 24 possible target carotenoids. The structure of these target carotenoids is represented in Fig. 2.

The assessment of the complexity and the success of a possible synthetic route to a carotenoid require an experienced chemist. The accessibility also strongly depends on the availability of precursors utilized in the outline of the synthesis of the target compound. By applying this criterion, about one-third of the approximate 25 target compounds could be eliminated.

Whether a compound can finally be formulated successfully is, in most cases, not easy to assess. A good solubility in a common organic solvent is usually a good indication. But again, to guess the solubility of a compound knowing only the chemical structure, is not possible. Only an experiment with the synthesized material will finally provide the answer.

Via this rational screening, applying the four main selection criteria as listed in Fig. 1 (color, stability, ease of synthesis, and customer acceptance), we came up with some 24 possible target structures.

We started to synthesize the most accessible carotenoids in this series and after receiving the results from the foreseen applications (model drinks and others) with the formulated carotenoid, we let these results influence the future direction of our exploration through these 24 target molecules.

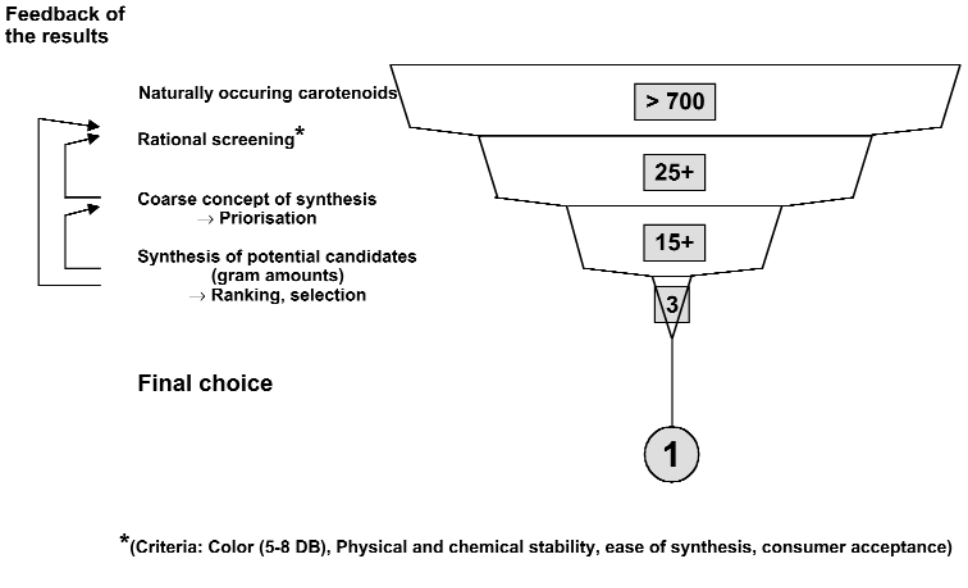

Fig. 1 Representation of the selection process via multistage elimination using different selection criteria.

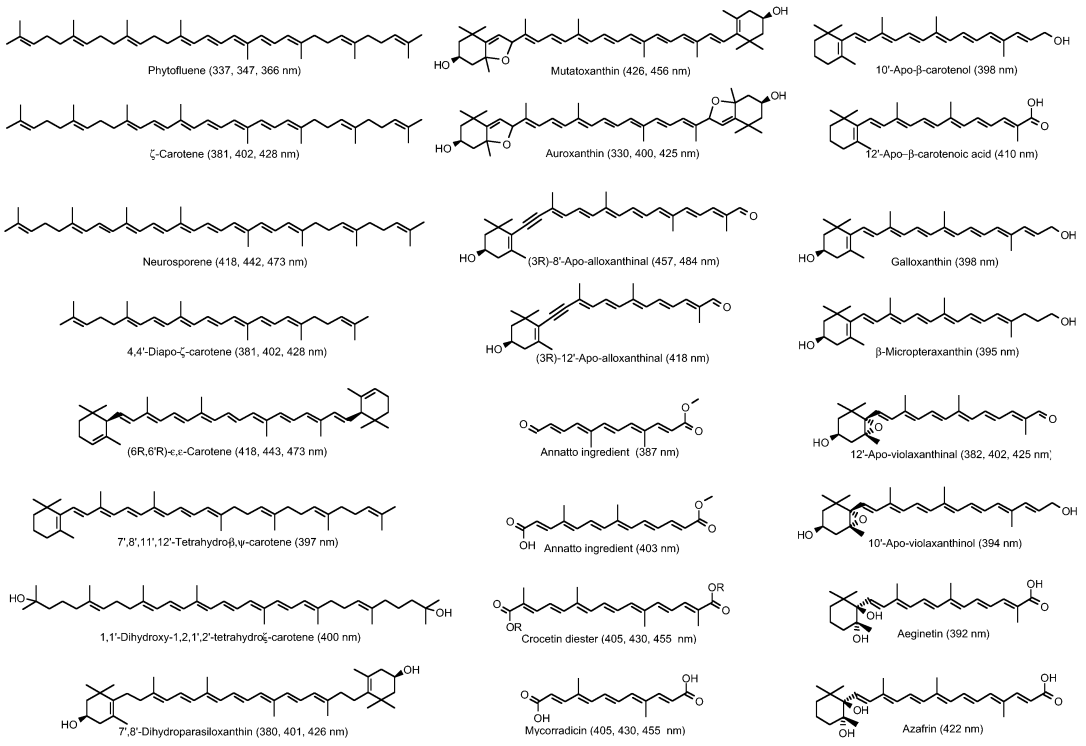

Fig. 2 Twenty-four possible carotenoid targets left after the first selection.

PROBLEMS WITH INTERPRETING Vis-SPECTRA

As mentioned earlier, the key to this project was to obtain a color that matches the target color. Unfortunately, in the literature, UV/Vis-spectra do not help to eliminate possible candidates due to various factors:

1. Vis-spectra of carotenoids sometimes do not only show one single absorption maximum but three. The open question remaining is which one of these maxima should be considered. Or is it more the overall shape of the Vis-spectrum that best represents the color of a formulated carotenoid in solution?
2. Often, the solvent in which the UV/Vis spectrum of a carotenoid was measured is not reported. Different solvents have a significant influence on the wavelength of the absorption maxima. For example, β,β-carotene exhibits in hexane a λ_{max} of 452 nm, in dichloromethane 462 nm, and in carbondisulfide 483 nm!
3. Formulation, the process that renders a nonwater-soluble carotenoid into a water dispersible form, has a drastic influence on the color appearance of a carotenoid dispersion. Roche sells, for example, several different forms of β,β-carotene for food coloration. One of these sales products, the so-called β,β-carotene 7 % cold water soluble (CWS), exhibits a nice fresh yellow (however, not with a green enough color hue like the targeted color sought as the goal in this project), whereas the β,β-carotene 10 % CWS sales product gives a "typical" β,β-carotene orange appearance when dissolved in water.

The conclusion of these remarks is that we have to synthesize all the possible candidates in sufficient amounts so that they can be formulated using a standard method and that the color of these formulations have to be compared to what we currently have in our product portfolio and also to the target color tartrazine.

In a third round, the most promising candidates will have to be synthesized in larger amounts. From this larger amount, several different formulations can then be prepared. These different formulations are then compared to each other and the target again.

The final selection of the candidate will then be done based on the same criteria as described in the section "Selection Process". Here, some prerequisites have already been assured, e.g., natural occurrence in the human food chain or ease of formulation, whereas other criteria will gain importance and have to be more carefully balanced, e.g., ease of synthesis (→ production cost) or color match.

SOME EXAMPLES OF THE SYNTHESIS OF CAROTENOIDS

As pointed out earlier, it is not possible to synthesize all of the more than 700 naturally occurring carotenoids cited in the *Key to Carotenoids* [1] and its supplement in *Carotenoids IA* [2]. The restrictions are mentioned in the section "Prerequisites for a Successful Candidate" help us to limit the number of possible target compounds to roughly 24 possible candidates. They are listed in Fig. 2 together with their λ_{max} recorded in petroleum ether as cited in *Carotenoids IB* [3].

We started to synthesize carotenoids that can be obtained easily through combining the most easily accessible precursors. If a more complex intermediate had to be synthesized, then this intermediate was used not only for this one carotenoid but, if possible for synthesizing several other candidates. An example for this is the synthesis of parasiloxanthin and 7,8-dihydroparasiloxanthin where a common precursor could have been used. The same holds true for ζ-carotene and neurosporene, galloxanthin and β-micropteraxanthin, 12′-apo-violaxanthin-12′-ol and 12′-apo-violaxanthin-12′-al.

In the following, the synthesis of a very limited selection of carotenoids will be presented.

Precursors of higher carotenoids

ζ-Carotene **1** and neurosporene **2** have the same structural motive on the right hand side of the chemical structure in common. This structural feature can be derived from (all-*E*)-farnesol **3**.

The readily accessible (*E*)-nerolidol **4**, which is used in large amounts in the fragrance and soap industry, can be converted to (all-*E*)-farnesol **3** following a procedure by Fujita et al. [4] (Fig. 3). From (all-*E*)-farnesol **3**, the C_{15}-Wittig salt **5** was obtained using various conditions. In a two-step procedure, farnesol **3** was first halogenated using a halogenation reagent (NBS, NClS, PBr_3, or similar) and from this C_{15}-halogen compound, the corresponding Wittig salt **5** was obtained after adding triphenylphosphine. Alternatively, the activating agent (HBr, HOAc, and other) could be combined with triphenylphosphine and (all-*E*)-farnesol **3**.

In an alternative route reported by Isler et al. [5], the C_{15}-bromide was synthesized when *E*-nerolidol **3** was reacted with PBr_3 in petroleum ether in the presence of pyridine. Then this bromide was reacted as described above.

The yield of this two-step process varied between 50 and 80 % depending on the reaction conditions.

From Wittig salt **5**, the symmetric ζ-carotene was obtained by a twofold Wittig condensation with the so-called C_{10}-dialdehyde **6**, (2*E*,6*E*)-2,7-dimethylocta-2,4,6-trien-1,8-dialdehyde) [6]. Using isopropanol as the solvent and sodium methylate as the base, a 69 % yield was achieved.

A Wittig condensation of apo-12′-lycopenal **7** [7] and the Wittig salt **5** in diethyl ether and dichloromethane with *n*-butyllithium as the base gave neurosporene **2** in 50 % yield.

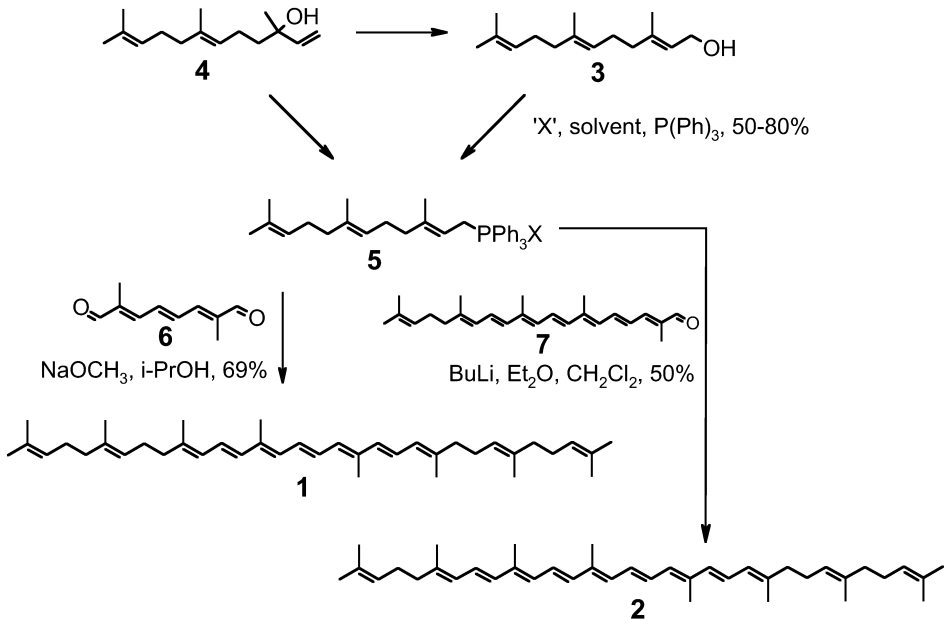

Fig. 3 Synthesis of ζ-carotene **1** and neurosporene **2** starting from *E*-nerolidol **4** or (all-*E*)-farnesol **3**.

Diapocarotenoids

In several organisms, diapocarotenoids are found as the main coloring matter. The different pigments of the pericarp of the Annatto fruits are an example of these diapocarotenoids. For a long time, these extracted pigments have been used to dye cheddar cheese, crackers, margarine, and many more foodstuffs. Another example, mycorradicin **10**, a bright yellow pigment, is formed in arbuscular mycorrhizal root fungi [8].

Even though root fungi do not belong to the human food chain, the diacid mycorradicin **10** was nevertheless synthesized because it could be obtained with simple and known synthetic steps (Fig. 4). C_{10}-Dialdehyde **6** was reacted in a twofold Horner-type reaction using the anion of triethyl phophonoacetate **8** (prepared by deprotonation with sodium hydride) in tetrahydrofuran (THF). Diester **9** was obtained in 75 % yield. Then **9** was saponified using an excess of 17 M aqueous sodium hydroxide in ethanol to give diacid mycorradicin **10** in 90 % yield.

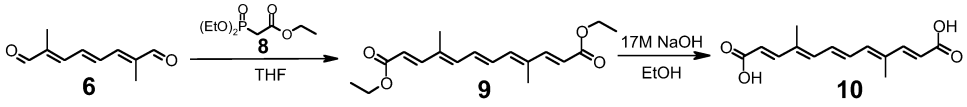

Fig. 4 Synthesis of mycorradicin **10**.

The ester acid diapocarotenoid **15** [(all-*E*)-4,8-dodeca-2,4,6,8,10-pentaendioic acid 12-methyl ester] was found by Scotter [9] as a concomitant of bixin and norbixin in Annatto extracts. The synthesis was carried out as shown in Fig. 5. The first step in this reaction sequence was the selective protection of the acid functionality as a *tert*-butyl-dimethylsilyl ester mediated by the weak base *N*-methylmorpholine. The primary allylic hydroxy group is then oxidized to the aldehyde. Horner-type condensation at this aldehyde functionality with the anion of trimethyl phophonoacetate and finally mild deprotection of the silyl ester under weakly alkaline condition in methanol yielded the desired

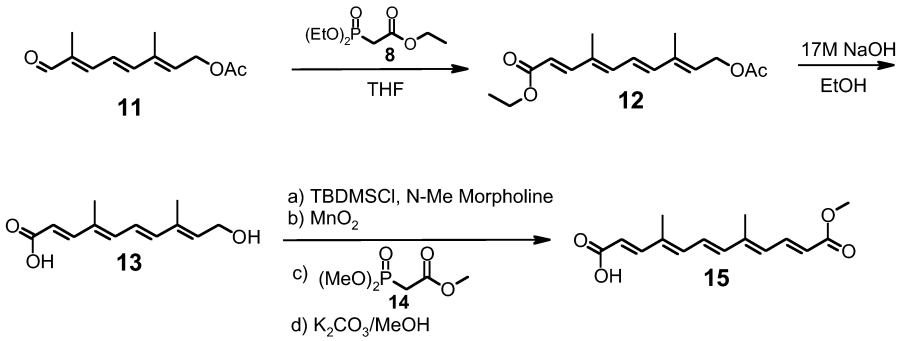

Fig. 5 Synthesis of the Annatto compound **15**.

diapo-carotenoid **15**. It is interesting to note that the four-step reaction from hydroxy acid **13** gave the target compound **15** in an excellent yield of 85 % and in a single reaction chamber.

Auroxanthin and mutatoxanthin

The two carotenoids, auroxanthin **16** and mutatoxanthin **17**, occur in the human food chain (e.g., Brussel sprouts, green peas, rose hips). They are presumably by-products of their parent structures violaxanthin **18** and antheraxanthin **19**, respectively, which are members of the xanthophyll cycle. Under slightly acidic conditions, violaxanthin **18** and antheraxanthin **19** can be converted to the furans auroxanthin **16** and mutatoxanthin **17**.

The synthesis of these two carotenoids started from (3*R*,3′*R*)-zeaxanthin **20** (Fig. 6). The secondary hydroxy groups of zeaxanthin were acetylated under standard conditions to give diacetate **21**

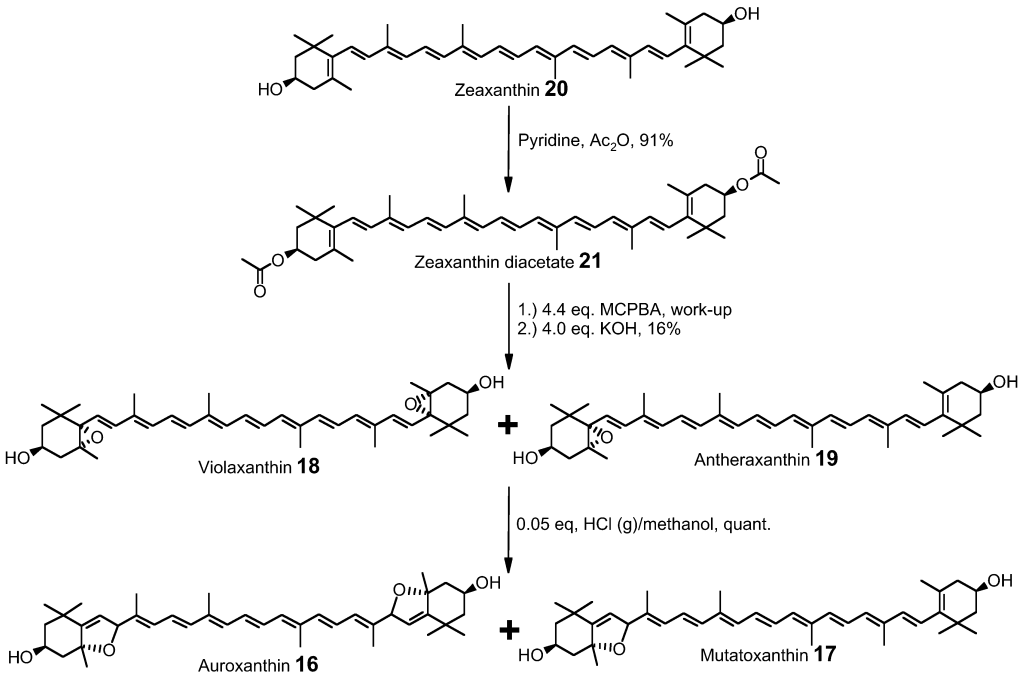

Fig. 6 Synthesis of auroxanthin **16** and mutatoxanthin **17**.

in 91 % yield. Epoxidation of the two cyclohexene olefins of diacetate **21** was achieved with metachloroperoxybenzoic acid (MCPBA) in dichloromethane. After work-up, the acetate was saponified. Since a complete formation of the epoxides at position 5,6 and 5′,6′ could not be accomplished, a mixture of violaxanthin **18** and antheraxanthin **19** resulted. The epoxide ring expansion to the furanoid systems under slightly acidic conditions in methanol was carried out in quantitative yield. As a consequence, only a diastereomeric mixture of auroxanthin **16** and mutatoxanthin **17** was obtained. This mixture was formulated and tested in food applications.

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