An Efficient Conversion of (3*R*,3′*R*,6′*R*)-Lutein to (3*R*,3′*S*,6′*R*)-Lutein (3′-Epilutein) and (3*R*,3′*R*)-Zeaxanthin[†]

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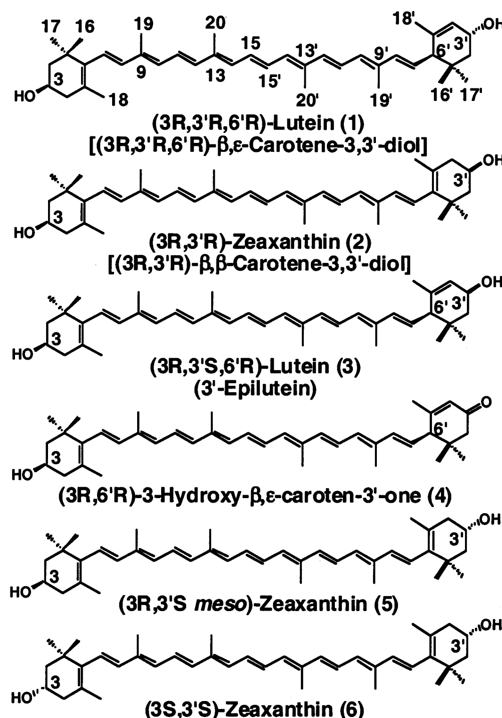
Two dietary carotenoids, (3*R*,3′*R*,6′*R*)-lutein (**1**) and (3*R*,3′*R*)-zeaxanthin (**2**), and their metabolite (3*R*,3′*S*,6′*R*)-lutein (3′-epilutein) (**3**) accumulate in human serum, milk, and ocular tissues. There is increasing evidence that compounds **1** and **2** play an important role in the prevention of age-related macular degeneration. Therefore, the availability of these carotenoids for metabolic studies and clinical trials is essential. Compound **1** is isolated from extracts of marigold flowers (*Tagete erecta*) and is commercially available, whereas **2** is only accessible by a lengthy total synthesis, and a viable method for synthesis of **3** has not yet been developed. This report describes an efficient conversion of technical grade **1** to **2** via **3**. Acid-catalyzed epimerization of **1** yields an equimolar mixture of diastereomers **1** and **3**. The mixture was separated by enzyme-mediated acylation with lipase AK from *Pseudomonas fluorescens* that preferentially esterified **3** and after alkaline hydrolysis yielded this carotenoid in 90% diastereomeric excess (de). Compound **3** was also separated from **1** in 56−88% de by solvent extraction and low-temperature crystallization, Soxhlet extraction, or supercritical fluid extraction. Base-catalyzed isomerization of **3** gave **2** in excellent yield, providing a convenient alternative to the total synthesis of this important dietary carotenoid.

A high intake of fruits and vegetables results in the absorption of a wide range of carotenoids and their metabolites by humans. These carotenoids have been fully characterized and quantified in human serum, milk, and tissues at varying concentrations.[1−3] Among these, two dietary carotenoids (xanthophylls), (3*R*,3′*R*,6′*R*)-lutein (**1**) and (3*R*,3′*R*)-zeaxanthin (**2**), accumulate at high levels in the human retina, particularly in the macular region.[4−6] Epidemiological and experimental studies have suggested that these carotenoids may protect the photoreceptors in the human retina from exposure to harmful short-wavelength blue light and prevent age-related macular degeneration (AMD) and other blinding disorders.[7,8] Further, nondietary (3*R*,3′*S*,6′*R*)-lutein (3′-epilutein) (**3**), (3*R*,6′*R*)-3-hydroxy-β,ϵ-caroten-3′-one (**4**), and (3*R*,3′*S* *meso*)-zeaxanthin (**5**) have been identified as the key metabolites of dietary **1** and **2** in human ocular tissues.[4−5,9] In view of the potential therapeutic application of **1** and **2** in the prevention of AMD, the development of industrial processes for production of these carotenoids has received considerable attention in recent years. The stereoselective total synthesis of **1** is associated with numerous steps and a poor yield,[10] and as a result, this carotenoid is isolated from saponified extracts of marigold flowers (*Tagete erecta,* variety *orangade*) and is commercially available as a nutritional supplement.[11] Although dietary **2** is very widely distributed in nature, its concentration in most readily available natural products is not sufficiently high for commercial production by extraction and isolation. Contrary to the situation with **1**, the stereoselective total synthesis of **2** has been accomplished by several lengthy and costly methods.[12] Because these procedures employ reagents that are toxic, the absence of possible residual contaminants in **2**, prepared by multistep synthesis, has to be established before this carotenoid can be safely used as a nutritional supplement or food coloring additive. There are also several processes that convert the commercially available **1** or crude saponified extracts of marigold flowers by base-catalyzed isomerization to optically inactive (3*R*,3′*S* *meso*)-zeaxanthin (**5**).[13−15] Although the *meso* form of zeaxanthin (**5**) is absent in foods and human serum, it has been identified as a metabolic product of dietary **1** in human ocular tissues.[4,9] In another process, **1** is transformed into **5** similar to the above methods, and the latter is oxidized to β,β-carotene-3,3′-dione followed by reduction with sodium or potassium borohydride to give a racemic mixture of **2**, **5**, and (3*S*,3′*S*)-zeaxanthin (**6**).[16] Due to the low overall yield and the fact that the racemic mixture of (3*RS*,3′*RS*)-zeaxanthin was not resolved, this approach



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does not provide an attractive route to the desired carotenoid **2**. Therefore, there is currently no efficient process that can convert **1** to **2** by a simple and economically viable process.

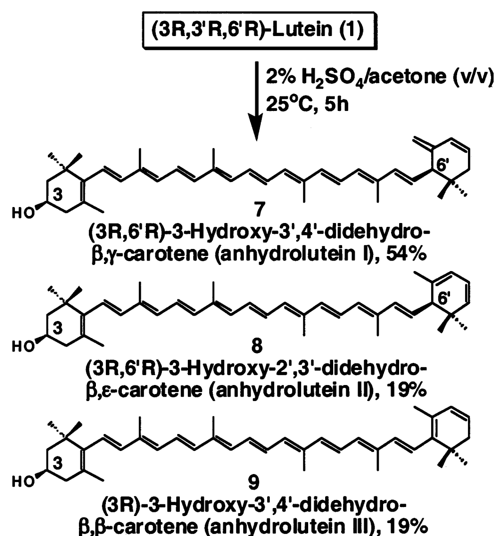
3′-Epilutein (**3**) is another carotenoid of interest that has been prepared by partial synthesis from reduction of (3*R*,6′*R*)-3-hydroxy-*β*,*ε*-caroten-3′-one (**4**) with diisobutylaluminum hydride (DIBAL-H).[17] However, this reduction is not stereoselective and gives a mixture of **1** and **3**. Because **3** is rare in nature and, similar to **1**, is difficult to prepare by total synthesis, a process for its partial synthesis from commercially available **1** is also highly attractive. This report describes a convenient and an industrially viable process for the conversion of **1** to **3** and further transforms the latter into **2** by base-catalyzed isomerization similar to a known method.[14]

Results and Discussion

There are two critical steps in the conversion of (3*R*,3′*R*,6′*R*)-lutein (**1**) to (3*R*,3′*R*)-zeaxanthin (**2**). These are (1) inversion of configuration at C-3′ in **1** and (2) isomerization of the isolated 4′ double bond in **1** to a conjugated double bond at position 5′. To take advantage of the superior reactivity of the allylic hydroxyl group at C-3′ in **1** relative to the hydroxyl group at C-3, the inversion of the configuration has to be accomplished prior to double-bond isomerization. Therefore, the transformation of **1** to **2** almost inevitably proceeds through **3**, providing convenient routes to both of these important compounds. Even though double-bond isomerization of **1** has been studied extensively, and as a result several patents for this reaction have been reported, an industrially viable method for the clean inversion of configuration of **1** at C-3′ has not been reported previously. Therefore, the existing technology converts **1** to **5** or transforms **1** into a racemic mixture of **2**, **5**, and **6**.[13−16] At present, there are two widely used methods for the inversion of stereochemistry of secondary chiral alcohols. The first method is known as the Mitsunobu reaction, which employs diethyl azodicarboxylate, triphenylphosphine, and an appropriate carboxylic acid to form a quaternary phosphonium salt that is then allowed to react with a secondary chiral alcohol to result in the inversion of configuration.[18] The Mitsunobu reaction has been previously used to convert **1** to **3** in a very low isolated yield.[19] The second method uses imidate esters as replacements for diethyl azodicarboxylate and triphenylphosphine in the Mitsunobu reaction.[20] This author has evaluated the application of both methods for the partial synthesis of **3** from **1**. It was found that the yield of **3** from the Mitsunobu reaction can be increased to about 20% under carefully dried reaction conditions, whereas the reaction of imidate esters with **1** resulted only in elimination of water and the formation of lutein dehydration products. Because of the low yield and the fact that diethyl azodicarboxylate is unstable and potentially explosive, the preparation of **3** from **1** by Mitsunobu reaction is not suitable for an industrial scale process.

One of the major difficulties in the total and partial synthesis of carotenoids is due to the sensitivity of the polyene chain of these compounds toward acid, heat, light, air, and harsh reagents that can cause side reactions such as *E*/*Z*-isomerization, oxidation, and degradation. Nonetheless, we have previously shown that, under controlled conditions, **1** undergoes acid-catalyzed dehydration with 2% H_2SO_4 in acetone (v/v) at ambient temperature to give a mixture of anhydroluteins **7**−**9** in excellent yields (Scheme 1).[21] On the basis of this observation, it was anticipated

Scheme 1. Acid-Catalyzed Dehydration of (3*R*,3′*R*,6′*R*)-Lutein



that if **1** was allowed to react with an acid in the presence of substantial amounts of water, formation of the dehydration products would be suppressed and the allylic hydroxyl group at C-3′ would undergo epimerization. This simple and convenient approach was expected to afford a diastereomeric mixture of **1** and **3** from which the latter carotenoid would have to be separated in a high de for this process to be industrially applicable.

To investigate this epimerization reaction, two grades of (3*R*,3′*R*,6′*R*)-lutein (**1**) were employed as the starting materials. These were commercially available **1** (technical grade) that contained approximately 70% total carotenoids (Kemin Foods, LC, Des Moines, Iowa) and a purified sample of (3*R*,3′*R*,6′*R*)-lutein (**1**) with 97% total carotenoids; the latter was prepared from technical grade **1** by recrystallization according to a published procedure.[22] Analysis of technical grade lutein by HPLC revealed the presence of minor quantities of several other carotenoids (ca. total of 8%), while purified lutein with 97% total carotenoid contained only approximately 5% of **2** (Table S1, Supporting Information).

When a solution of **1** (70% or 97% total carotenoid) in tetrahydrofuran (THF) was allowed to react with a dilute aqueous solution of either H_2SO_4, HCl, or H_3PO_4 at ambient temperature, an equimolar mixture of diastereomers **1** and **3** was obtained almost quantitatively. HPLC studies of the course of this reaction showed that, depending on the nature and concentration of the acid, the equilibrium between **1** and **3** was established after nearly 12−17 h. The carotenoid compositions of 70% and 97% pure lutein and their products of acid-catalyzed epimerization in dilute aqueous acid were determined by HPLC on a silica-based nitrile-bonded column, and the data are shown in Table S1 (Supporting Information). The minor carotenoids that were present in technical grade lutein, for the most part, remained unchanged upon treatment with dilute acid. Because of the non-allylic nature of the hydroxyl groups in **2**, this carotenoid did not react with acid and was fully recovered. However, approximately 1% of anhydroluteins **7**, **8**, and **9** were formed and 1−2% of **1** underwent in-chain isomerization to a mixture of its *Z*(*cis*)-isomers. For a typical HPLC profile of the products of acid-catalyzed epimerization of **1** (97% pure) see Figure S1 (Supporting Information). It is imperative to point out that the HPLC separations should be carried out on a silica-based nitrile-bonded column with spherical particles. This is because

nitrile-bonded columns in which particle shape was not spherical did not separate these carotenoids. Some of the minor carotenoids could not be separated on a nitrile-bonded column, and as a result their relative distribution was determined by HPLC on a C_{18} reversed-phase column according to a previously published procedure.[2] Analytically pure samples of **1**, **2**, and **3** were separated from the crude products by semipreparative HPLC and identified from their UV/visible, mass, and ^{1}H NMR spectra based on authentic samples derived from our previously published procedures.[23] Minor carotenoids including anhydroluteins **7**–**9** and *Z*-luteins were similarly identified by comparison of their spectroscopic data with those of authentic samples prepared previously.[21–23]

It was discovered that 3′-epilutein (**3**) has a greater solubility than (3*R*,3′*R*,6′*R*)-lutein (**1**) in nonpolar hydrocarbon solvents such as pentane, hexane, heptane, cyclohexane, and petroleum ether. Thus, a number of processes were developed that allowed partial to almost complete separation of these epimers. These were (a) solvent extraction and low-temperature crystallization, (b) Soxhlet extraction, (c) supercritical fluid extraction using carbon dioxide, and (d) enzyme-mediated acylation. When each of these methods was separately applied to an equimolar mixture of **1** and **3**, the latter was obtained in 54–90% diastereomeric excess (de). Enzyme-mediated acylation was found to be the most suitable method and afforded **3** in 90% de, whereas the other methods resulted in only partial separations.

Although **3** exhibits a greater solubility in hydrocarbon solvents than **1**, both are only sparingly soluble in nonpolar solvents. For example, the solubility of **3** in hexanes was approximately 8 mg/100 mL. Consequently, for partial separation of diastereomers **1** and **3** by extraction, large volumes of a hydrocarbon solvent are needed, which is not practical in scale-up operations. This problem can be overcome by the use of a cosolvent in which **1** and **3** have increased solubility. A combination of an ether [diethyl ether, *tert*-butyl methyl ether (TBME)] and a hydrocarbon (pentane, hexane, heptane, petroleum ether) was found to be most effective. When a crystalline equimolar mixture of **1** and **3** was stirred with a mixture of an ether and a hydrocarbon at ambient temperature for 30 min, after filtration, **3** was obtained in 56% de. Slightly better separation of these carotenoids was achieved when EtOH was used as the extracting solvent. The results of these experiments are summarized in Table S2 (Supporting Information). Since the separation of **1** and **3** by EtOH extraction at ambient temperature was promising, the partially separated mixtures were further subjected to low temperature (−20 °C) crystallization with this solvent to increase the de of **3**. For example, the mother liquor from low-temperature crystallization of a partially separated mixture of **3** (86%) and **1** (14%) was shown by HPLC to consist of 94% of **3** and 6% of **1**.

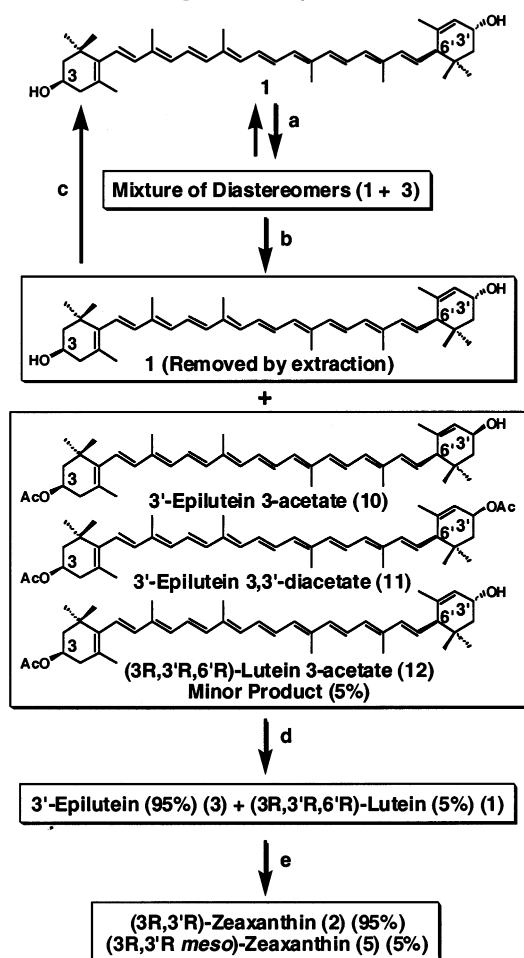
Soxhlet extraction of an equimolar mixture of **1** and **3** with a nonpolar hydrocarbon, such as pentane, for 4–5 h resulted in partial separation of these carotenoids. When Soxhlet extraction was performed with hexane or heptane, the separation was poor and significant amounts of *Z*-isomers of **1**–**3** were found in the hydrocarbon-soluble fractions. The best results were obtained by extraction with pentane or petroleum ether, and after 4 h, more than 90% of **3** was extracted from the mixture by these solvents. These extracts were shown by HPLC to consist of **3** (77%), **1** (19%), total *Z*-luteins (2%), and anhydrolutein (2%). The remaining solid consisted of **3** (20%), **1** (70.5%), and **2** (9.5%). *Z*-Luteins and anhydroluteins (**7**–**9**) that were present in the starting material were completely extracted into the hydrocarbon-soluble fraction, while **2** remained in the solid phase.

Partial separation of **1** and **3** was also accomplished using supercritical fluid extraction (SFE) with carbon dioxide. When an equimolar mixture of **1** and **3**, containing minor quantities of **2**, was subjected to SFE, it was revealed that the solubility of **3** in carbon dioxide was far greater than that of **1** and **2**. Small-scale extraction of the above carotenoid mixture (5 mg) with 150 g of carbon dioxide at 300–350 atm resulted in extraction of 85% of the **3**. The composition of carotenoids in the extracted fraction was as follows: **3** (83%), **1** (13%), *Z*-luteins (2%), and anhydroluteins (2%). Therefore after excluding the minor carotenoids the relative distribution of **3** and **1** was 86.5% and 13.5%, respectively, corresponding to 73% de for **3**. On the basis of the difference in solubility behavior of **1** and **3**, the use of a hydrocarbon (hexane, heptane) or an alcohol (ethanol, methanol) modifier with SFE would be expected to facilitate the extraction of **3** in large-scale operations.

Irreversible, enzyme-mediated resolution of secondary allylic alcohols in organic solvents employing lipase AK from *Pseudomonas fluorescens* and lipase PS from *Pseudomonas cepacia* have been well documented in the literature.[24–27] To this author's knowledge, there is no example in the literature that has documented the separation of diastereomeric chiral carotenoid alcohols by enzyme-mediated acylation. In the absence of selectivity data that could correlate specific lipases to structural features of carotenoids, a number of commercially available lipases were arbitrarily screened to identify the lipase suitable for the separation of **1** and **3**. Among these, only lipase AK from *Pseudomonas fluorescens* and lipase PS from *Pseudomonas cepacia* were found to promote the acylation of **3** in organic solvents (pentane, hexane, TBME) in the presence of vinyl acetate (acyl donor) at 36 °C. The reaction was best performed in refluxing pentane. Under these conditions, 3′-epilutein was esterified to give 3′-epilutein 3-acetate (**10**) and 3′-epilutein 3,3′-diacetate (**11**) as major products, while (3*R*,3′*R*,6′*R*)-lutein 3-acetate (**12**) was obtained as minor product (≈5%) (Scheme 2). It was interesting to note that the HPLC studies of the course of the reaction on a C_{18} reversed-phase column (eluent B) revealed that 3′-epilutein is first esterified at the position 3 to form the monoester **10**, which then undergoes further acylation at position 3′ to the diester (**11**) (Figure S2, Supporting Information). Because the monoester **10** and the diester **11** are highly soluble in hydrocarbon solvents, these were effectively separated from **1** by solvent extraction at 0 °C; the recovered **1** can be recycled into the epimerization step. Monoester **12** (≈5%) was also removed with the 3′-epilutein esters **10** and **11** by solvent extraction. After alkaline hydrolysis of the mono- and diesters **10**–**12**, approximately 95% of **3** (90% de) and 5% of **1** were obtained. For identification purpose, the crude product from one of the lipase-mediated acylation reactions was subjected to flash column chromatography followed by preparative HPLC on a nitrile-bonded column to obtain analytically pure samples of the esters **10**, **11**, and **12**. The structure of the esters were then established from their ^{1}H NMR, mass, and UV/visible spectra.

When the enzymatic acylation was attempted on the diastereomeric mixture of **1** and **3** prepared from 70% pure lutein, no reaction was observed. This presumably was due to the presence of non-carotenoid impurities that were probably carried over in the epimerization and lipase-

Scheme 2. Overall Sequence for Synthesis of **3** and **2** from **1**[a]



[a] (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 (pentane, hexane, petroleum ether) to remove carotenoid esters; (d) KOH/MeOH/THF, 25 °C; (e) base-catalyzed isomerization, KOH/phase transfer catalyst, hexane, 64 °C, 5 h.

mediated acylation steps. Therefore, prior to enzymatic acylation, the mixture of **1** and **3** prepared from 70% pure lutein should be purified by flash column chromatography on a silica gel column to remove these non-carotenoid impurities. Alternatively, if the diastereomeric mixture of **1** and **3** was prepared from the 97% pure **1** and the products are then subjected to enzymatic acylation, the reaction proceeded smoothly and no additional purification was needed.

In a typical experiment, a mixture of **1** and **3**, prepared from 97% pure **1**, was acylated with vinyl acetate in the presence of lipase AK or PS at 36 °C in pentane or hexane. After 48 h with lipase AK approximately 5% of **3** remained unreacted. Lipase PS catalyzed this reaction much more slowly than lipase AK, and after 72 h, 10% of **3** was found unesterified. At the end of these reactions, THF was added to solubilize all the carotenoids, the precipitated enzyme was removed by filtration, and the product was evaporated to dryness. The residue was washed with pentane or hexane at 0 °C to remove esters **10**−**12** as well as minor quantities of anhydroluteins **7**−**9**. The hydrocarbon-insoluble solids consisted of **1** and minor quantities of **2** that was present in the starting material. After hydrolysis of the esters **10**−**12** with alcoholic potassium hydroxide, the product consisted of mainly **3** as well as minor quantities of **1** (≈5%) and anhydroluteins **7**−**9** (≈2%). The acylation of **3** mediated by lipase AK and lipase PS, after alkaline hydrolysis, afforded this carotenoid in 90% and 70% de, respectively. While TBME could also be employed as solvent with these enzymes with almost identical results as those observed with pentane and hexane, the acylation of **3** did not proceed in THF.

The overall sequence for transformation of (3*R*,3′*R*,6′*R*)-lutein (**1**) to (3*R*,3′*R*)-zeaxanthin (**2**) is shown in Scheme 2. In the final step of this sequence, **3** was subjected to base-catalyzed isomerization to yield **2**. At the outset, one would expect this isomerization to proceed quite readily because of the acidity of the hydrogen at C-6′ and the fact that the migration of the isolated double bond at C-4′ into conjugation with the polyene chain of **3** would yield a more thermodynamically stable carotenoid. In all of the reported cases, prolonged heating of **1** with a base at temperatures between 70 and 120 °C in an organic solvent was needed to promote the isomerization to **5**. However, at elevated temperatures, a considerable amount of *E*/*Z*-isomerization of **1** and **5** takes place that is undesirable. The Hoffmann-La Roche patent has solved this problem by conducting the isomerization in a two-phase system with an aqueous solution of an alkali hydroxide and a phase transfer catalyst in a saturated aliphatic and/or aromatic hydrocarbon solvent at temperatures ranging from 50 to 120 °C.[14] Therefore, this procedure was employed to promote the isomerization of **3** to **2**. A suspension of **3** (95%) and **1** (5%), separated by lipase AK acylation, was treated with a 11.5 M aqueous solution of KOH in refluxing hexanes (68 °C), employing tricaprylylmethylammonium chloride (Aliquat 336) as a phase transfer catalyst. After 5 h of reflux, **3** and **1** were completely converted to **2** (95%) and **5** (5%), respectively, and no significant *E*/*Z*-isomerization of these carotenoids was observed by HPLC.

In summary, this report describes an efficient synthesis of 3′-epilutein (**3**) and (3*R*,3′*R*)-zeaxanthin (**2**) from commercially available (3*R*,3′*R*,6′*R*)-lutein (**1**) in several convenient steps.[28] In the first step, **1** is epimerized at C-3′ in THF with an aqueous acid at ambient temperature to give a diastereomeric mixture of **3** and **1**, almost quantitatively. During the workup of the crude product from this reaction, an appropriate organic solvent can be employed to separate **3** in 50−54% de. The poorly separated mixtures of **1** and **3** can then be subjected to low-temperature (−20 °C) crystallization with EtOH to obtain the latter in up to 88% de. Several other methods have also been developed that separate **3** from **1**. These are Soxhlet extraction, supercritical fluid extraction with carbon dioxide, and lipase-mediated acylation. While some of these methods result in poor separation of **3** from **1**, low-temperature crystallization and lipase-mediated acylation are the most effective and afford **3** in 88−90% de. The results of the various methods that have been employed to separate **3** from **1** are summarized in Table S4 (Supporting Information). In industrial-scale operations, a simplified combination of these procedures can be employed to obtain **3** in a high de. For example, the crude products from epimerization of **1** can be simply enriched in **3** during the workup by solvent manipulation. The resulting diastereomeric luteins, enriched in **3**, can then be subjected to Soxhlet extraction followed by low-temperature crystallization to further separate this carotenoid from **1** in a high de. The final transformation of **3** to **2** may be accomplished by base-catalyzed isomerization similar to a published procedure.[14]

Experimental Section

General Experimental Procedures. Technical grade (3*R*,3′*R*,6′*R*)-lutein (70% pure) was obtained from Kemin Foods (Des Moines, Iowa) and was further purified to 97% by

crystallizations according to published procedures.[11,22] Lipase AK from *Pseudomonas fluorescens* ("Amano" 20) (activity 28 887 U/g) and lipase PS from *Pseudomonas cepacia* ("Amano") (activity 31 000 U/g) were obtained from Amano Enzyme U.S.A. Co., Ltd. (Lombard, IL). Vinyl acetate, tricaprylylmethylammonium chloride (Aliquat 336), and *N,N*-diisopropylethylamine (DIPEA) were obtained from Aldrich Chemical Co. (Milwaukee, WI); all other commercial grade and HPLC grade solvents were used without further purification.

HPLC analyses were performed on an Agilent Technology model 1100 HPLC system equipped with a quaternary solvent delivery system, 1100 autosampler, thermostated column compartment, and 1100 diode array detector. Details regarding analytical and semipreparative HPLC separations are described in the Supporting Information.

The mass spectra of carotenoid acyl esters were obtained by FAB on a JEOL SX102a mass spectrometer. The matrix was "m-b", magic bullet, consisting of a 5:1 mixture of dithiothreitol/dithioerythritol. Employing this technique, the parent molecular ions of carotenoid acyl esters appeared as radical cations and not as $(M + H)^+$ ions. The mass spectra of all other carotenoids were obtained by interfacing the HPLC system into a Hewlett-Packard model 5989A particle beam mass spectrometer (HPLC-MS). Eluate from the HPLC was divided with a ratio of 1:3, with the lesser amount entering the particle beam interface that was operated at a desolvation temperature of 45 °C. Electron capture negative ionization (ECNI) was achieved using methane at a pressure of 0.85 Torr and a source temperature of 250 °C. Spectra were collected from *m*/*z* 100 to 700 using a scan cycle time of 1.5 s. The ^{1}H NMR spectra were recorded in $CDCl_3$ on a Bruker AMX-400 spectrometer (400 MHz), and the chemical shifts are referenced to $CHCl_3$ (7.26 ppm). The UV/visible absorption spectra were obtained by HPLC-photodiode array detection between 200 and 600 nm at the rate of 12 spectra per minute; the UV/visible spectra of the isolated carotenoids were also obtained in single solvents on a Beckman UV/visible spectrophotometer model DU-530.

Epimerization of 70% Pure (3*R*,3′*R*,6′*R*)-Lutein (1). Technical grade (3*R*,3′*R*,6′*R*)-lutein (7 g, 70% total carotenoids) in THF (600 mL) was stirred with 250 mL of an aqueous solution of HCl (0.3 N, pH ≈ 0.56 at 23 °C) at ambient temperature under nitrogen. The course of the reaction was followed by HPLC. After 12 h, an aqueous solution of 5% sodium bicarbonate was slowly added, and the crude product was partitioned into 300 mL of *tert*-butyl methyl ether (TBME) containing 1% (v/v) triethylamine. Other organic solvents such as diethyl ether or ethyl acetate were also used with similar results. The aqueous layer was removed, and the organic layer was washed with water and dried over Na_2SO_4. The solvent was evaporated under reduced pressure below 40 °C to give a mixture of epimerized luteins **1** and **3** and several minor carotenoids as orange crystals (6.7 g).

For purification and characterization of carotenoids, 3 g of the crude product was subjected to flash column chromatography on n-silica gel (150 g, 60−200 mesh) using 4:1 hexanes/acetone, and two main colored bands were collected. The first band (yellow) consisted of a number of minor carotenoids, which were identified by comparison of their HPLC retention times and UV/visible and mass spectral data with those of authentic samples as α-cryptoxanthin, β-cryptoxanthin, anhydroluteins **7**−**9**, and β-carotene similar to published procedures.[1,2] The second band (red) was shown by HPLC−UV/visible−MS on a silica-based nitrile-bonded column to consist of **1**, **2**, **3**, and minor quantities of anhydroluteins **7**−**9**.[1,2,23] The following carotenoids were isolated by semipreparative HPLC on a silica-based nitrile-bonded column and were fully characterized from their UV/visible, HPLC-MS, and ^{1}H NMR spectra.

(*all-E*,3*R*,3′*R*,6′*R*)-Lutein (1): UV/visible (hexane) λ_{max} 330 (444 main max), 473 nm; HPLC-MS spectrum (ECNI, methane), molecular anion peak at *m*/*z* 568 (100%) and an anion peak at *m*/*z* 550 [20%, $(M - H_2O)^-$] (calcd for $C_{40}H_{56}O_2$); ^{1}H NMR data were in agreement with literature values.[10,23,29−32]

(*all-E*,3*R*,3′*R*)-Zeaxanthin (2): UV/visible (hexane) λ_{max} 338 (451 main max), 477 nm; HPLC-MS spectrum (ECNI, methane), molecular anion peak at *m*/*z* 568 (100%) and an anion peaks at *m*/*z* 550 [55%, $(M - H_2O)^-$] and *m*/*z* 532 [15%, $(M\text{-}2H_2O)^-$] (calcd for $C_{40}H_{56}O_2$); ^{1}H NMR data were in agreement with literature values.[23,30−32]

(*all-E*,3*R*,3′*S*,6′*R*)-Lutein (3′-epilutein, 3): UV/visible (hexane) λ_{max} (444 main max), 473 nm; HPLC-MS spectrum (ECNI, methane), molecular anion peak at *m*/*z* 568 (20%) as well as anion peaks at *m*/*z* 550 [100%, $(M - H_2O)^-$] and *m*/*z* 532 [60%, $(M - 2H_2O)^-$] (calcd for $C_{40}H_{56}O_2$); ^{1}H NMR data were in agreement with literature values.[23,29−32]

Partial Separation of 3 from 1 by Solvent Extraction. An equimolar mixture of **1** and **3** (0.1 g) was suspended in *tert*-butyl methyl ether (3 mL) and hexane (9 mL) in a centrifuge tube. The mixture was stirred at room temperature for 30 min, and the tube was centrifuged. The solid was removed and dried under high vacuum to give dark orange crystals (54 mg) of a mixture of **1** (64%), **3** (19%), **2** (10%), and other minor carotenoids (7%). The solution was evaporated to give 46 mg of a dark red residue, which was shown by HPLC to consist of a mixture of **3** (73%), **1** (21%), **2** (1%), and other minor carotenoids (5%).

Separation of 3 from 1 by Soxhlet Extraction. An equimolar mixture of **1** and **3** (0.1 g) obtained from epimerization of a 97% pure **1** was placed in a thimble (50 × 10 mm) inside a Micro-Soxhlet extractor. The mixture was extracted with boiling pentane (bp = 36 °C) for 4 h. Pentane-soluble carotenoids (51 mg) consisted of **3** (77%), **1** (19%), total *Z*-luteins (2.0%), and anhydrolutein (2.0%). Approximately 49 mg of the carotenoids remained as solids in the thimble. This was shown by HPLC to consist of **3** (20.0%), **1** (70.5%), and **2** (9.5%). Nearly identical results were obtained when extraction was carried out with petroleum ether (bp = 35−60 °C).

Separation of 3 from 1 by Low-Temperature Crystallization. A partially separated mixture of **3** (86%) and **1** (14%) (50 mg) containing approximately 2% of other minor carotenoids was dissolved in 7 mL of EtOH in a centrifuge tube. The solution was kept at −20 °C for several hours until **1** crystallized. The tube was centrifuged, and the supernatant solution was removed and evaporated to dryness to give 21.3 mg of a red solid; this was shown by HPLC to consist of **3** (94%) and **1** (6%). The EtOH-insoluble fraction was dried under high vacuum to give 27.5 mg of an orange solid of **1** (19%), **3** (79%), and **2** (2%).

Separation of 3 from 1 by Supercritical Fluid Extraction. An equimolar mixture of **1** and **3** (5 mg) was mixed with 0.70 g of hydromatrix (mixture of silica gel and diatomaceous earth). This was extracted with carbon dioxide in a supercritical fluid extraction apparatus, Model Prep-Master (Suprex-ISCO, Inc., Lincoln, Nebraska). The conditions were as follows: flow = 2 mL/min, restrictor temperature = 50 °C, desorb temperature = 10 °C, oven temperature = 35 °C. A two-step gradient was employed for extraction of **3**. Step 1 used 100 g of carbon dioxide at 300 atm and step 2 50 g of carbon dioxide at 375 atm. A total of 2.4 mg of carotenoids was extracted with this 150 g of carbon dioxide. The extracted carotenoids were shown by HPLC to consist of **3** (83%), **1** (13%), anhydroluteins (2%), and *Z*-luteins (2%). Approximately 2.5 mg of carotenoids remained in the hydromatrix; these were **1** (77%), **3** (13%), and **2** (10%).

Separation of 3 from 1 by Enzyme-Mediated Acylation with Lipase AK. A mixture of **1** and **3** (0.1 g) was suspended in pentane (20 mL). Lipase AK (30 mg) from *Pseudomonas fluorescens* ("Amano" 20) and vinyl acetate (50 μL) were added, and the mixture was heated under reflux (36 °C) for 48 h under an atmosphere of nitrogen. After this time, according to HPLC approximately 5% of **3** had remained unesterified. The crude product was shown by HPLC to consist of **1** (40%), **3** (5%), **10** (23%), **11** (21%), **12** (5%), **2** (5%), and anhydroluteins (**7**−**9**) (1%). THF (15 mL) was added and stirring continued for 5 min to dissolve all the carotenoids. The enzyme was removed by filtration, and the filtrate was evaporated to dryness. The residue was stirred with pentane (30 mL) at 0 °C for 30 min and filtered. The solids were washed with cold pentane (10

mL) and dried under high vacuum to give 51.2 mg of a mixture of **1** (86%), **3** (4.7%), and **2** (9.3%). The pentane-soluble fraction was shown by HPLC to consist of **10** (49%), **11** (45%), **12** (5%), and anhydroluteins (1%). The solvent was evaporated, and the pentane-soluble fraction was dissolved in THF (10 mL) and treated with 10 mL of methanolic KOH (10%). The mixture was stirred at room temperature for an hour, and the product was partitioned between H_2O (20 mL) and *tert*-butyl methyl ether (20 mL). The H_2O layer was removed, and the organic layer was washed with H_2O (2×10 mL), dried over sodium sulfate, and evaporated to dryness. This gave 48.3 mg of a dark red solid, which was shown by HPLC to consist of **3** (94.5%), **1** (4.5%), and **7** (1%).

The following carotenoid esters were purified by semipreparative HPLC on a nitrile-bonded column and were identified from their UV/visible, MS, and ^{1}H NMR spectra.

3′-Epilutein 3-acetate (10): UV/visible (hexane) λ_{max} (444 main max), 473 nm; FABMS, molecular radical cation at *m/z* 610 (100%) (calcd for $C_{42}H_{58}O_3$); ^{1}H NMR data were in agreement with literature values; see the Supporting Information.[30,32]

3′-Epilutein 3,3′-diacetate (11): UV/visible (hexane) λ_{max} (444 main max), 473 nm; 18); FABMS, molecular radical cation at *m/z* 652 (100%) (calcd for $C_{44}H_{60}O_4$); ^{1}H NMR data were in agreement with literature values; see the Supporting Information.[30,32]

(*3R,3′R,6′R*)-Lutein 3-acetate (12): UV/visible (hexane) λ_{max} 330 (444 main max), 473 nm; FABMS, molecular radical cation at *m/z* 610 (100%) (calcd for $C_{42}H_{58}O_3$); ^{1}H NMR data were in agreement with literature values; see the Supporting Information.[30,32]

Base-Catalyzed Isomerization of 3 to 2. A mixture of **3** (95%) and **1** (5%) (100 mg) was suspended in hexane (10 mL) and was treated with 100 mg of tricaprylylmethylammonium chloride (Aliquat 336) and 5 mL of 11.5 M KOH. The mixture was heated at 64 °C under nitrogen, and the reaction was followed by HPLC employing eluent A. After 5 h, the mixture was cooled to room temperature and the product was stirred with 10 mL of MeOH for 5 min. The suspension was filtered, and the solid was washed with MeOH (3×10 mL) and dried under high vacuum. The solid was crystallized from CH_2Cl_2 and hexane at −20 °C to give 85 mg of a mixture that was separated by HPLC on a chiral column according to a published procedure;[9] carotenoids in the mixture were identified as **2** (95%) and **5** (5%).

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Supporting Information Available: Details regarding analytical and semipreparative HPLC separation and HPLC profiles of compounds **1**−**12**. Tables S1−S4. ^{1}H NMR data for compounds **10**−**12**. This material is available free of charge via the Internet at http://pubs.acs.org.

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