Recent progress in commercial retinoids and carotenoids

J. Paust
BASF Aktiengesellschaft, Central Research Laboratory, 6700 Ludwigshafen, Germany

Abstract - Among the retinoids vitamin A has an exceptional position both by volume and in its field of application. The competing technical syntheses are discussed. The other retinoids namely (all E)- and (13 Z)-Retinoic acid and Etretinate are used as topical and systemic agents in dermatology.

Commercial synthetic carotenoids are mainly used as pigments for food in vivo; examples are egg yolk - and broiler pigmentation with ß-apo-ß'-carotenonic acid ethylester and pigmentation of farm-raised salmon with astaxanthin. Another field of application is direct coloration of food, e.g. of margarine with ß-carotene. Presently ß-carotene is booming as a substitute for vitamin A without teratogenic potential and as a biological antioxidant. Two strategies compete in manufacture of these carotenoids:

- synthesis of ß-apo-ß'-carotenoids and ß-carotene starting from vitamin A and its precursors followed by the stepwise oxidation of ß-carotene to canthaxanthin and astaxanthin
- conversion of oxo-isophorone to C₁₀-phosphonium salts of different oxidation pattern in the ß-ionone ring followed by Wittig condensation with C₁₀-dialdehyde.

INTRODUCTION

This report deals with the retinoids and carotenoids which are currently commercially available. It is in the nature of things that information on these cannot be very accurate or complete. The manufacturers of these products are competitors and thus not interested in revealing details of syntheses or capacities and manufacturing costs.

The two groups of products to be dealt with are summarized in Fig. 1 and 2. In total, four retinoids and six synthetic carotenoids have to date become of commercial importance.
Among the retinoids, vitamin A occupies a special position. It is the most important product in terms of quantity sold. Vitamin A is mainly used in the feed- and foodstuffs sector: it ensures that livestock thrive and increases their resistance to infectious diseases (ref. 1).

The other retinoids are used in pharmaceuticals and employed in dermatology (ref. 2).

The main reason for producing the carotenoids is for their coloring properties; they are used on the one hand for direct coloring of foodstuffs and, on the other hand, for pigmentation of animal products by administration in the feed (ref. 3). In addition, it is becoming increasingly evident that the carotenoids have important roles in the animal and human body, besides their provitamin A activity (ref. 4).

**VITAMIN A**

Three companies are currently marketing vitamin A - Hoffmann-La-Roche, BASF AG and A.E.C. Rhône-Poulenc, and they produce about 3000 tonnes of this vitamin in total each year. In the form of a 15 % dispersible powder in which it is usually sold, 1 kg of vitamin A presently costs about US $ 120. The sales worldwide are therefore about US $ 360 million.

The syntheses used by the three manufacturers differ first in the basic chemicals used to synthesize pseudoionone and 6-ionone, secondly in the type of intermediates used for chain extension and finally in the method of linkage employed to give the C20 final product.

Fig. 3. Synthesis of 6-ionone from basic chemicals.

Pseudoionone is currently prepared from dehydro-linalool or citral (ref. 5, 6). Both precursors can be synthesized in several steps from basic chemicals, with sigmatropic rearrangements playing a crucial part (Fig. 3). A fascinating example is the double Cope rearrangement of the dienol ether that is produced by acetalization and elimination from 3-methylbutenal (Fig. 4, ref. 7).

The C6 and C8 units employed for chain extension are also used to build up the polyene chain of carotenoids. The basic chemicals for the C6 unit at Hoffmann-La-Roche are methyl

Fig. 5. Syntheses of 2-methyl-4-acetoxy-2-butenal (C5 acetate).
vinyl ketone and acetylene (ref. 8). The C₁₅ unit used by Rhône-Poulenc is prepared from 3-methyl-1-butyn-3-ol via 3-methyl-2-buten-1-ol (ref. 9). At BASF the C₁₅ acetate is obtained by hydroformylation of 1-butene diacetate (Fig. 5, ref. 10) or by a synthesis based on oxirane and acetic acid via acetoxyacetaldehyde and condensation with propionaldehyde (ref. 11). Selective aldol condensations of this type give yields around 70% in the presence of equimolar amounts of dimethylamine and acetic acid; the catalyst system can be recycled.

The polyene chain must be built up under particularly mild conditions. Hoffmann-La-Roche and Rhône-Poulenc achieve this by addition and elimination, the former by linking C₁₃ and C₅ in a Grignard reaction (ref. 12) and the latter by Julia-synthesis from C₁₅ and C₅ (ref. 13). At BASF AG, C₁₅ is joined to C₅ in a one-step Wittig reaction (ref. 14, Fig. 6).:

It is possible that, in future, syntheses which do not take place via the C₁₃ intermediates pseudoionone and β-ionone will become important. Kuraray in Japan has done intensive work on the preparation of a cyclic C₁₀ sulfone and an open-chain C₁₀ aldehyde from myrcene and linalool respectively (ref. 15). The polyene chain is produced by linking the two units and preparing a β-alkoxy or 6-chloro-C₁₀-sulfone by base-promoted double elimination.

Vitamin A syntheses based on the C₉ + C₆ scheme have not, to my knowledge, achieved any economic importance as yet (ref. 16). This synthetic approach is mentioned here nevertheless, because the C₉ + C₆ strategy has proven very useful in carotenoid syntheses.

Over the years the synthesis of the "immortal" agent vitamin A has been modified repeatedly. As shown in Fig. 7 new processes have been developed for the production of the C₁₅- and C₅-precursors due to the availability of cheap basic chemicals.

Thus in 1960, 8 out of the 20 carbon atoms of vitamin A originated from acetylene and 6 from diketene or ethylacetoacetate (ref. 17). The remaining 6 carbon atoms stem from acetone. Thirty years later the situation has changed completely: 8 carbon atoms can be traced back to isobutene and 4 carbon atoms to formaldehyde (ref. 5, 6 and 10); acetone contributes 3 carbon atoms in positions 8, 9 and 19.
RETINOIDS

In contrast to vitamin A, which is an additive for human and animal foodstuffs, the other retinoids are pharmaceuticals (ref. 2). They have revolutionized the treatment of various skin disorders in the last decade. There has not yet been a breakthrough in tumor therapy, although inhibition of progression has been detected in animal experiments (ref. 18).

Retinoic acid (Tretinoin) is used topically for the treatment of acne but it is also becoming increasingly important for the treatment of skin damaged by UV-light (ref. 19). (13-Z)-Retinoic acid (Isotretinoin) can be used systemically, and this has made it possible for the first time to cure serious acne (ref. 20). Estimated sales in 1989 are US $150 Mill. for Tretinoin and 100 Mill for Isotretinoin. The aromatic retinoid Etretinate has proven especially useful as a systemic agent for the treatment of inherited disorders of keratinization and certain types of psoriasis (ref. 21).

![Diagram of retinoic acid synthesis](image)

Fig. 8. Synthesis of Retinoic acid and (13 Z)-Retinoic acid

The retinoids which have been approved to date are synthesized in substantially the same way as vitamin A (Fig. 8, ref. 22). For example, (all E)- and (13 Z)-retinoic acid are obtained straightforwardly and in good yields from the C15-phosphonium salt and C5-aldehyde esters or the appropriate C5-lactol. The oxidation of retinyl acetate to retinoic acid with silver oxide has been described and is preferred by manufacturers who do not have the C15 and C5 units available (ref. 23).

Fig. 9 gives a forecast of the future uses and market volumes of retinoids. World sales may exceed US $1 billion in the 1990s. The prospects of this have recently led to a considerable intensification of research in this area. The main problems associated with this are well known in pharmaceutical research, namely (1) - Selection of actions from the almost bewildering variety displayed by retinoids. These include effects on cell differentiation, cell proliferation, sebum production and keratinization, in addition to immunostimulation, antiinflammatory effects and morphogenic activities (2) - Improvement of the therapeutic index by reducing undesired side effects, in particular reduction of the potential for irritation on topical application and reduction of the teratogenicity on systemic administration. With the availability of water-soluble β-glucuronide derivatives, problems of formulation and of absorption and transport of retinoids are overcome (ref. 24). Pelretin, which is a phenyl analog, has been shown in clinical tests to have a particularly low potential for irritation (ref. 25). The tetramethyltetralin derivatives are expected to have the most favorable spectrum of action to date for the treatment of sun-damaged skin (ref. 26).

Research into these questions now even extends into molecular biology. There is intensive investigation of the effects of retinoids on gene expression. Various retinoid acid receptors have been identified, and it is now possible to synthesize retinoids that are designed to fit these (ref. 27).
COMMERCIAL SYNTHESIS OF CAROTENOIDS

Carotenoid synthesis is still the preserve of Hoffmann-La-Roche and BASF AG, where vitamin A is manufactured, partly because from the outset these companies had the required range of precursors and experience in synthesizing polyene chains. Both companies initially concentrated on constructing the relatively large target molecules by using carbon-carbon linkage methods that were already proven in vitamin A chemistry; these were, in particular, (1) at Hoffmann-La-Roche, Grignard syntheses and enol ether condensations (ref. 9, 28) and (2) at BASF AG, ethynylation and Wittig olefin syntheses (ref. 12, 14). This type of synthetic strategy permits the processes to be simplified and makes multistage syntheses economical.

β-APO-CAROTENOIDS

The strategy is evident in the preparation of the β-apo-8'-carotenoids and β-carotene. The synthesis of β-apo-8'-carotenal at Hoffmann-La-Roche starts from the C14 aldehyde which, in two enol ether condensations, the C16 unit is followed by another two enol ether condensations to give the C20 final product (ref. 29). The related β-apo-8'-ester is produced by condensation of the precursor C27, aldehyde with the appropriate Wittig C3 ylde (ref. 30).

For BASF the obvious route was to prepare these carotenoids by using the Wittig reaction. When the double bonds are formed in position 11, 15 and 11', chemical yields > 90 % of (E/Z)-isomers are obtained. Isomeric purity of the final products is controlled by conversion of the sterically hindered (Z)-components to the (all-E)-products with simultaneous crystallisation.

The β-apo-8'-carotenoids can be built up by C20 + C5 Wittig reactions; both companies have reported this (ref. 31). The yields are comparable when retinal is linked to a C10 phosphonium salt or when retinyl triphenyl phosphonium salt is linked to the appropriate C10 aldehyde.

Syntheses based on the C20 + C5 scheme appear short and elegant, but the preparation of the unsymmetrical C10 fragments is a multistage and thus costly process. It is therefore advantageous to prepare the β-apo-8'-carotenoids via β-apo-12'-carotenal. Three equally efficient pathways have been developed for the synthesis of this intermediate. These are Wittig reactions of (1) C10 phosphonium salt with the vitamin A synthesis with one equivalent of the unsymmetrical C12 dialdehyde (ref. 32), (2) C10 phosphonium salt with 2-methyl-butenenal-1-acetal (ref. 33, Fig. 11) and (3) retinal with the corresponding C10 phosphonium chloride (ref. 34, Fig. 12). Retinal is obtained either by dehydrogenation of retinol with oxygen and TEMPO/CuCl as catalyst (ref. 35) or by Wittig reaction of the C10 phosphonium salt with another selectively protected C5 dialdehyde, 3-methyl-butenenal-1-acetal (ref. 33, Fig. 12).

The C20 aldehyde and C30 ethyl ester target compounds are obtained from the C20 aldehyde by Wittig reaction with the C5 phosphonium salt (ref. 34) or with an appropriate C5 ethyl ester phosphonate (ref. 36).
Fig. 11 and 12. Retinal and β-apo-12′-carotenal in the synthesis of β-apo-8′-carotenoids

C5-INTERMEDIATES

3-Methyl-butenedial-1-acetal is easily obtained in two stages via glyoxal monoacetal by selective aldol condensation with propanal (ref. 33).

The C5 ester component is obtained from 4-bromo-2-methyl-2-butenoi acid ethyl ester and triethyl phosphite. The bromo-ester itself is synthesized (1) from methyl vinyl ketone and hydrocyanic acid via 2-methyl-2-hydroxy-3-butenoi acid ethyl ester (ref. 37) by reaction with phosphorous tribromide or (2) from 2-methyl-3-butenonitrile via the ethyl ester by addition of bromine and elimination of hydrogen bromide (ref. 38).

It is particularly economical to prepare all the C5 components from the C5 acetate intermediate that is used in the vitamin A process (Fig. 13).

Oxidation of the C5 acetate with persulfuric acid in ethanol gives 4-hydroxy-2-methyl-2-butenoi acid ethyl ester in 80% yield. This is converted to the C5 ethyl ester phosphonate in an obvious way.

The C5 alcohol obtained by acetalization with a 1,3-diol and ester hydrolysis can be converted by reaction with phosgene and triphenyl phosphine into the C5 phosphonium chloride in 80% overall yield.

2-Methyl-butenedial-1-acetal is obtained by dehydrogenation of the C5 alcohol with oxygen and the TEMPO/CuCl2 catalyst in 90% yield. Other polyene allylic alcohols can also be converted into the aldehydes in this way (ref. 35). On Wittig reaction with the C5 phosphonium chloride and hydrolysis the new C5 aldehyde gives a 90% yield of the symmetrical C10 dialdehyde which is the central part of many C40 carotenoids.
Recent progress in commercial retinoids and carotenoids

To complete the group of β-apo-carotenoids of commercial interest, the synthesis of the C₃₃ ketone citranaxanthin should be mentioned briefly. It is produced in about 90% yield from β-apo-β'-carotenal by aldol condensation with acetone (ref. 39).

As the name indicates, the β-apo-carotenoids are formed by oxidative degradation of the C₄₀ carotenoids, especially of β-carotene. As such they have always been present in our food and thus they are absolutely non-toxic in the natural concentrations. They are, therefore, highly suitable natural colorants for foods (ref. 1, 3). β-Apo-C₄₀ ester (ref. 40) and citranaxanthin are used in the pigmentation of egg yolks and broilers, especially when the feedstuffs contain few natural xanthophylls. β-Apo-C₃₀ aldehyde is used for direct coloring of foodstuffs, especially cheese. The total sales of this group of products are now about US $50 million.

β-CAROTENE

β-Carotene has been produced by Hoffmann-La Roche since 1954 and by BASF since 1972. As with the β-apo-carotenoids, the syntheses of β-carotene in both companies lean heavily on the range of vitamin A precursors.
Hoffmann-La Roche linked 2 moles of the C$_2$H$_2$ aldehyde which has already been mentioned with acetylene bis(magnesium halide) and obtained B-carotene after partial hydrogenation and acid-catalyzed elimination of 2 moles of water (ref. 41).

BASF AG linked two moles of the C$_2$H$_2$ phosphonium salt precursor for vitamin A to the symmetrical C$_2$H$_2$ aldehyde (ref. 42), or linked retinal to the C$_2$H$_2$ phosphonium salt obtainable from vitamin A (Fig. 15, ref. 43). Both routes have been used industrially, depending on the availability of the precursors, and, after thermal isomerization, provided crystalline (all-E)-β-carotene in yields above 80%.

These classical Wittig syntheses of β-carotene can be further simplified by assembling the molecule from two identical halves in one reaction step. Thus, β-carotene was obtained in yields of about 80% by McMurry (ref. 44) who reduced retinal with titanium(II) compounds, and by Bestmann (ref. 45) who oxidized retinylidenetriphenylphosphorane with the ozone adduct of triphenylphosphate. We found that β-carotene is obtained more easily and in good yield when the oxidation of the C$_2$H$_2$ phosphonium salt is carried out with hydroperoxides in alkaline medium. The best results are obtained with potassium carbonate and hydrogen peroxide in water at ambient temperature. Removal of triphenylphosphine oxide by extraction and thermal isomerization result in analytically pure β-carotene in yields of around 80% (ref. 46).

There is an economic advantage in the use of the C$_2$H$_2$ phosphonium salt in the synthesis of carotenoids (Fig. 16): crystallization of (all E)-retinyl acetate leads to mother liquor which contains considerable quantities of (E/Z)-isomeric vitamin A compounds. These become useful only after they have been converted with triphenylphosphine and sulfuric acid into the phosphonium salt, mainly because it is then possible to remove impurities straightforwardly by washing with nonpolar solvents. The costs of raw materials for β-carotene are therefore determined essentially by the price of triphenylphosphine; β-carotene has thus become the lowest-cost carotenoid.

When Wittig olefin syntheses are carried out on the industrial scale, as BASF AG has been doing for about 30 years, it is an advantage to have a good source of triphenylphosphine. The process BASF AG has developed is based on reaction of chlorobenzene with liquid sodium in toluene followed by addition of phosphorus trichloride (ref. 47). This makes extreme demands on process control and safety.

The oldest use for β-carotene is the direct coloring of foodstuffs. Various preparations have been developed for this. A microcrystalline dispersion in an edible fat is used in margarine manufacture. For aqueous media, for example for coloring fruit juices, powders in which β-carotene is in the form of a microdispersion in a hydrophilic protective colloid are used.

β-Carotene is always present in chlorophyll-containing plants and is thus the most widely distributed carotenoid. Its role as a cofactor in photosynthesis is now well understood. Carotenoids and, in particular, β-carotene appear to carry out functions in animals and humans which go far beyond acting as an optical signal and having provitamin A activity. For example, β-carotene enhances immunity and acts as an intracellular antioxidant to supplement tocopherol which mainly acts in the cell membrane (ref. 4). These findings point to important new uses of β-carotene, especially since it is entirely nontoxic. β-Carotene is already used in livestock for enhancing fertility and is now an ingredient of many vitamin products for human use. Terms like "chemoprevention" in precancerous states and "radio protection" in conjunction with radiation treatment of cancer and "early prophylaxis in cardiovascular diseases" have been coined (ref. 49).
The developments outlined here are also reflected in the current and predicted sales of β-carotene. It is likely that B-carotene will soon overtake vitamin A. Capacity for more than 500 tonnes of β-carotene per year is currently planned or under construction worldwide. B-Carotene sales may reach US $500 million towards the end of this century. Total sales of synthetic carotenoids are already in the order of US $300 Mill. and may pass the US $500 million mark in only about 5 years.

THE DEVELOPMENT OF CANTHAXANTHIN AND ASTAXANTHIN

In the 1950s it was discovered at Hoffmann-La-Roche that some of the canthaxanthin added to chicken feed is deposited in the egg yolk (ref. 50). Together with the xanthophylls which are naturally present or with B-apo-C20 ster, canthaxanthin gives the egg yolk an orange-red color which many consumers like. Carotenoids in the feed are also deposited in the bodies of poultry, being evident in the comb, beak and legs. This is not simply a cosmetic effect. It is now known that carotenoids enhance resistance to diseases and improve the shelf life and hatchability of eggs.

Commercial processes for the preparation of canthaxanthin are based on the oxidation of β-carotene and are thus developments of the very early investigations by Zechmeister and Karrer (Fig. 17, ref. 51). The principle is that β-carotene is halogenated in position 4 and then, via intermediates from solvolysis reactions, oxidized to canthaxanthin. In our variant of the process, β-carotene is oxidized with sodium chlorate in the presence of catalytic amounts of iodine. Yields of about 65% of isolated (all E)-canthaxanthin are obtained in a dichloromethane-water two-phase system (ref. 52).

Hoffmann-La-Roche subsequently took the initiative and synthesized canthaxanthin by the tried and tested C15 + C10 + C15 scheme (Fig. 18, ref. 53). The novel 3-oxo-C15 phosphonium salt can be prepared by the C13 + C1 and C2 + C6 routes. In both cases, the components are linked by addition of an organometallic compound to a carbonyl group. The C10 unit can be obtained, for example, by selective reduction of the 4-oxo group to 4-hydroxy-6-oxo-isophorone (ref. 54); canthaxanthin yields of around 80% are obtained from reaction of the C15 ylide with the C10 dial and the intermediate C25 aldehyde.
It is known that wild salmon acquire astaxanthin and its esters from their food and store the pigment in their tissues. As aquaculture, especially of salmon, has intensified in recent years, there has been a rapid increase in interest in this carotenoid (ref. 55).

Synthetic astaxanthin has been marketed only by Hoffmann-La-Roche to date, and this company is responsible for all serious approaches to the synthesis of this carotenoid.

Astaxanthin can, in principle, be obtained by oxidation of other C30 carotenoids, for example from canthaxanthin by preparing the bis-silyl enol ether and oxidizing this with peracid (Fig. 19, ref. 56, 59). The protective groups prevent further oxidation to astacene.

Commercial astaxanthin synthesis uses the familiar C15 + C10 + C15 scheme. The C15 unit is once again produced by the C9 + C6 route, and the C15-acetone is synthesized in three straightforward steps from oxo-isophorone (ref. 57). The same principle is used, via a C9 unit prepared by fermentation, to synthesize (3R,3'S)-zeaxanthin (Fig. 20, ref. 58).

**SYNTHETIC STRATEGIES**

To summarize, there appear to be two fundamentally different strategies for the synthesis of these carotenoids (Fig. 21 and 22). On the one hand, β-carotene can be synthesized from vitamin A and triphenylphosphine and then oxidized specifically to canthaxanthin and astaxanthin. On the other hand, it is possible to prepare a specific trimethylcyclohexanone for each carotenoid (ref. 59). Alkylation with the C9 component and conversion of the C15 unit into the phosphonium salt can be followed by Wittig reaction with the C10 dialdehyde to give the final products. This strategy is attractive because it is self-contained and, in principle, also permits the synthesis of vitamin A and the essential vitamin E precursor trimethylhydroquinone (ref. 60).

The two synthetic approaches allow certain conclusions to be drawn about the costs of manufacturing these carotenoids. It is possible to deduce from the "vertical" scheme that, although β-carotene should be accessible at very low cost from vitamin A, the more highly oxygenated carotenoids must be increasingly costly. By contrast, the "horizontal" scheme starting from oxo-isophorone is expected to have comparable costs of manufacture of the individual carotenoids at comparable levels of production.
Recent progress in commercial retinoids and carotenoids

Current market prices for the synthetic carotenoids discussed above in the form of stabilized dispersible powders containing 5 - 10 % active substance are in the order of US $ 600 for β-carotene, US $ 900 for the β-apo-8'-carotenoids, US $ 1300 for canthaxanthin and US $ 2600 for astaxanthin. It is evident that the present price structure is still determined by a "vertical" synthetic strategy.

WATER-SOLUBLE PREPARATIONS

The fact that the syntheses are efficient and that the prices of these products are not low may give the impression that manufacturers of carotenoids are making ridiculous profits. The high prices of the carotenoids are particularly determined by the costly step from the pure lipophilic substances to the products developed for use in aqueous media. In order to develop fully the potential color strength or to achieve a high level of bioavailability in the gastrointestinal tract, the coarse crystalline material has to be transformed into a microdisperse state with an average particle size of about 0.1 - 0.2 µm.

Mechanical means are not, in general, successful for achieving this. For example, suspensions obtained by grinding in the presence of an edible oil have a particle size distribution in the range 1 - 5 µm. Therefore, an alternative route that is commonly employed is micronization based on a combined dissolution/precipitation process.

The micronization process developed at BASF AG is based on water-miscible and non-toxic solvents like acetone or ethanol. A suspension of the carotenoid in the selected solvent is fed into mixing chamber I (Fig. 23, ref. 61) where it is rapidly mixed with an appropriate volume of the pure solvent.

Appropriate choice of the delivery rate of each pump makes it possible to control the residence time, the temperature of mixing, and the final concentration of the active ingredient.
As it turns out, a residence time of about 0.5 sec at 170 °C in mixing chamber I is adequate for obtaining a molecular solution from a β-carotene suspension with an average particle size of about 20 μm.

The molecular solution emerging from mixing chamber I is fed into mixing chamber I1 where it undergoes turbulent mixing with a cold aqueous solution of a protective polymer, resulting in precipitation of the carotenoid. Appropriate choice of the polymer makes it possible to limit the growth of the particles, after nucleation, to a size of about 0.1 μm. A dispersible powder can be obtained from the hydrosol by conventional methods, for example spray-drying, after the solvent has been removed by distillation.

Summing up it was shown how commercial carotenoids are produced starting from cheap basic chemicals and how these pure crystalline compounds are adapted to the different fields of application by sophisticated physicochemical operations. This results in a collection of toxicologically safe and high-quality food and feed-additives.

REFERENCES


Recent progress in commercial retinoids and carotenoids


40. US Pat. 2 940 856 (1960).
42. Ger. Pat. 924 247 (1956), 1 068 703 (1959), 1 068 705 (1959), all to BASF AG.
44. The Regents of the Univ. of California (Berkeley/CA-USA), Ger. Pat. 2 515 011 (1975).