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# Terpenoids

## Carotenoids

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### 1. GENERALITIES

The carotenoid group includes several hundred tetraterpenoid compounds consisting of a sequence of eight isoprene units. Their characteristic chromophore—at least ten conjugated double bonds—explains their yellow or orange color and their extreme tendency to get oxidized.

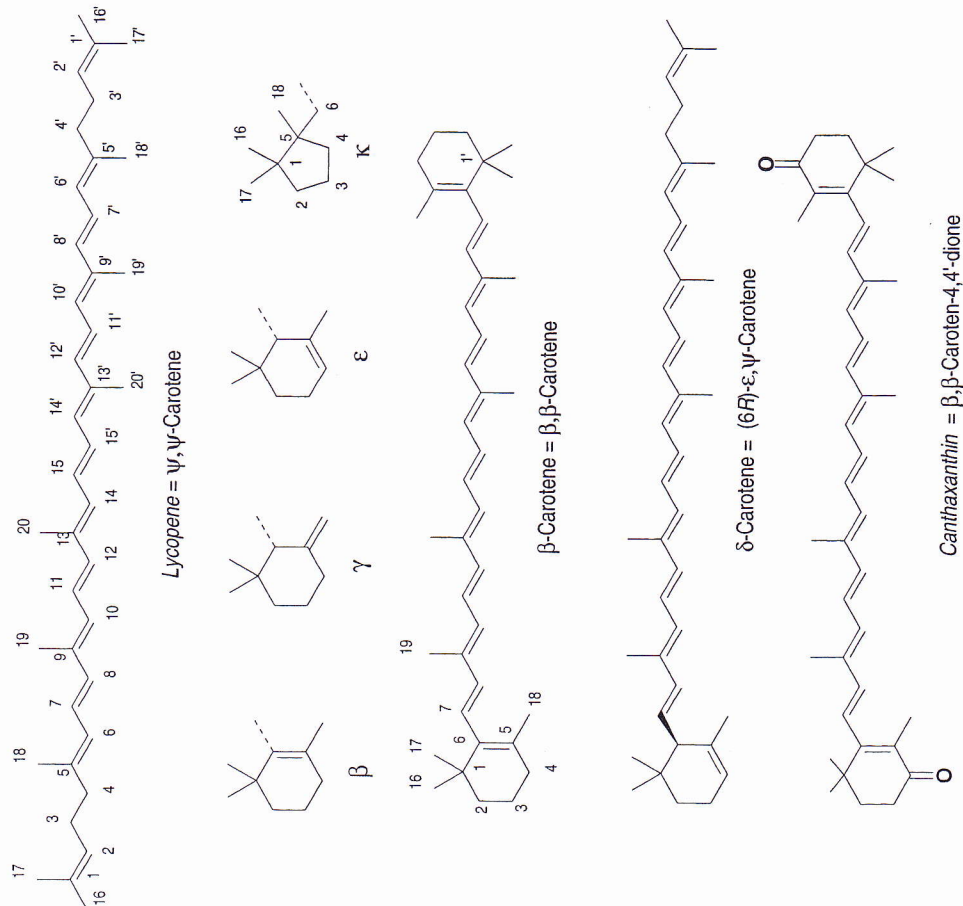
Among those compounds, the hydrocarbons are collectively referred to as *carotenes*, and the hydroxylated derivatives as *xanthophylls*. Since there are multiple unsaturations, there could be multiple geometrical isomers: in reality, most carotenoids have a poly-*E* configuration. Carotenoids are either acyclic (e.g., lycopene), or comprise one or two penta- or hexacyclic rings at one end or the other (e.g.,  $\beta$ , $\psi$ -carotene), or at both ends (e.g.,  $\beta$ , $\beta$ -carotene).

To facilitate the naming of these compounds (whose common name is often inspired by their botanical origin), it is customary to 1. start from the basic collective name and structure, e.g., carotene; 2. number the carbons corresponding to the basic

carotene skeleton from the two ends of the molecule to the middle (i.e., 1,2,3...15 and 1',2',3'...15'); 3. identify the structure at each end of the molecule by the proper Greek letter (there are seven possibilities: not cyclized [ $\psi$ ], cyclopentanoid [ $\kappa$ ], cyclohexenoid [ $\beta$ , $\epsilon$ ], cyclohexanoid [ $\gamma$ ], or—but these compounds are specific to the photosynthetic bacteria—benzenoid [ $\phi$ ,  $\chi$ ]); 4. identify the various substituents. For example, lycopene is  $\psi$ ,  $\psi$ -carotene,  $\beta$ -carotene is  $\beta$ ,  $\beta$ -carotene,  $\delta$ -carotene is (6*R*)- $\epsilon$ ,  $\psi$ -carotene, and capsanthin is (3*R*,3',5,5'*R*)-3,3'-dihydroxy- $\beta$ , $\kappa$ -caroten-6'-one.

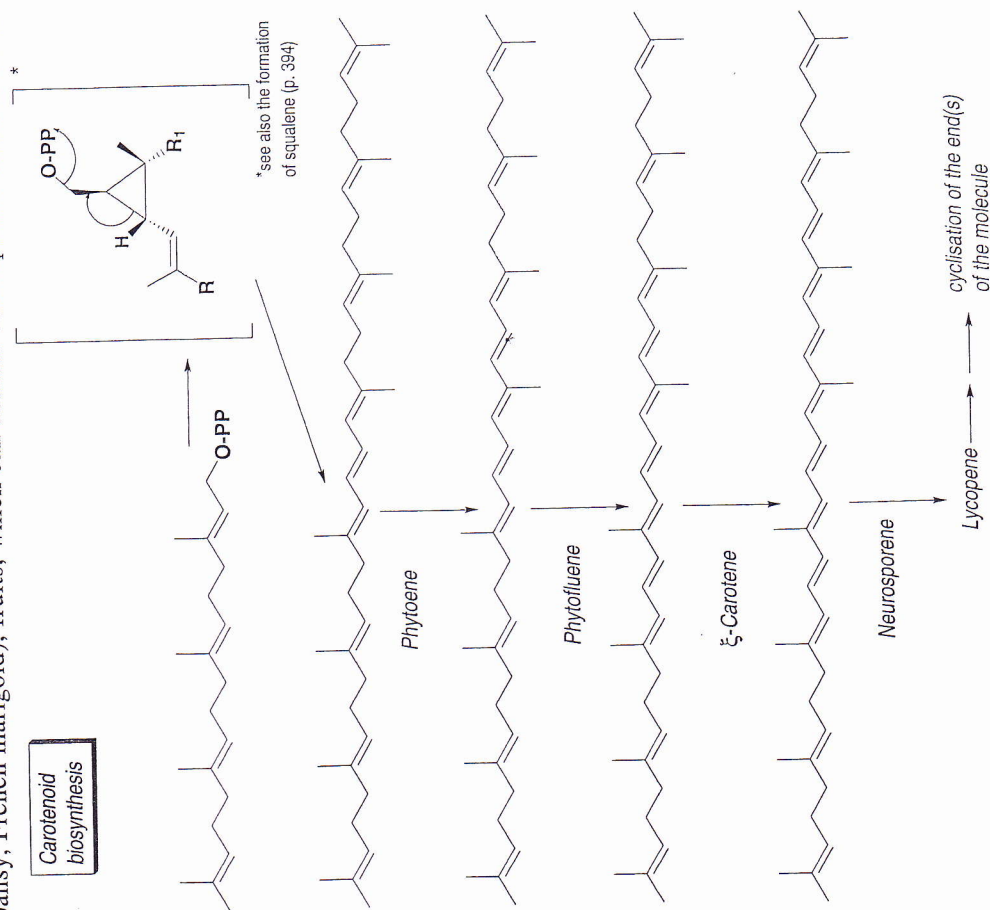
## 2. BIOSYNTHETIC ORIGIN, DISTRIBUTION, AND FUNCTIONS

Biosynthetically, carotenoids are formed by a mechanism analogous to that which leads to squalene: the "tail-to-tail" condensation of two molecules of geranylgeranyl pyrophosphate results in a cyclopropane-type intermediate, namely prephytoene

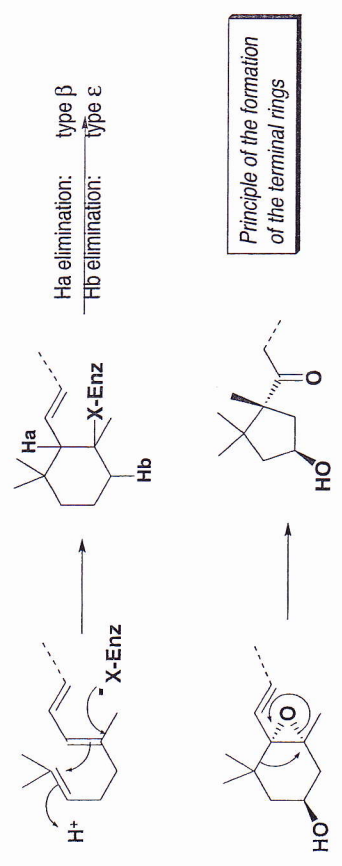


pyrophosphate. Unlike for presqualene pyrophosphate, the carbonium ion is not stabilized by protonation by NADPH, but by the loss of a proton, and a 15,15'-unsaturated hydrocarbon is formed: phytoene. Phytoene then undergoes stepwise unsaturation to phytofluene,  $\xi$ -carotene, neurosporene, and finally, lycopene. The unsaturated end(s) may cyclize as a result of the protonation of the terminal double bond, and subsequently, the products may be oxidized (at C-3), epoxidized, or even partially degraded (apocarotenoids, carotenic acids). The  $\kappa$ -type cyclopentane moiety arises from the rearrangement of a 5,6-epoxy- $\beta$ -carotene. Note that a few non-photosynthetic bacteria elaborate C<sub>45</sub> and C<sub>50</sub> carotenoid homologs.

These pigments occur widely. They accumulate in the chloroplasts of all of the photosynthetic tissues:  $\beta$ -carotene, lutein, violaxanthin, and neoxanthin occur in the leaves of almost all plants. Carotenoids also accumulate in flower petals (marigold, pansy, French marigold), fruits, which can contain chloroplastic carotenoids or



accumulate other derivatives (capsanthin of capsicum, apocarotenoids of *Citrus*, lycopene in tomato), roots (carrot: carotenes), and seeds (corn: zeaxanthin) where their concentration may reach high levels (anatto). They are also found in Fungi, although this is rather uncommon. Carotenoids occur in the protein complexes of the photosynthetic systems of the thylakoid membrane, and are involved in photosynthesis: their polyalkene system with its delocalized electrons allows them to absorb and transmit blue-green radiation (450-500 nm). They also (and mostly?) provide protection against harmful radiation (by reacting with singlet oxygen, thereby preventing photo-oxidation). Because their double bonds are conjugated, these compounds are very efficient scavengers of the singlet oxygen produced during photochemical processes. They can also act as antioxidants: they react with the peroxy radical, thereby preventing its addition onto polyalkenes and the subsequent production of oxygenated compounds.



### 3. INTEREST IN CAROTENOIDS AND USES

The interest in carotenoids has multiple justifications.

- $\beta$ -carotene and closely related compounds are degraded, at the level of the human intestinal mucosa, to retinol (vitamin A): this contributes to the value of some fruits and vegetables (carrots, spinach, mangos, tomatoes, bell peppers, squash, *Citrus*, melons), and also some animal products (eggs, liver, fish), or palm oil (margarine). The vitamin A activity of  $\beta$ -carotene is 1,600,000 IU/g (1 IU of vitamin A corresponds to 0.3  $\mu\text{g}$  of retinol and to 0.6  $\mu\text{g}$  of  $\beta$ -carotene).
- Carotenoids are thought to exert a preventive action against degenerative disorders. The results of many epidemiological studies designed to assess the relationship between the consumption of fruits and vegetables containing—among others—carotenes (or carotene ingestion) and different cancers suggest, in an aggregate sense, that dietary carotenoids might have a protective role against various cancers. There is clearly an inverse relationship between blood levels of carotenes and the risk of lung cancer, but for all other cancers, the results are inconclusive; on

the relationship between the incidence of cancer and carotenoid-rich foods, the results are mixed, and they show that there is no relationship between the incidence of cancer and plasma levels of  $\beta$ -carotene.

In the past few years, several experiments were conducted on humans with daily carotene administration for several years: except for one study carried out in China, they showed that  $\beta$ -carotene has no beneficial effect on the incidence of malignant neoplasms. On the contrary, in the case of lung cancer, two studies showed an increased mortality in subjects at risk (smokers, former smokers\*) upon supplementation with  $\beta$ -carotene. These intervention studies and other studies also failed to confirm the relationship that some authors had suspected between increased carotene intake and decreased cardiovascular disease morbidity. Although there is no justification for recommending a regular carotene intake, experts agree that these negative results do not cast any doubt on the benefits of a diet rich in fruits and vegetables.

- Carotenoids, because they interfere with photo-oxidation processes, may be of use in the treatment of photosensitization linked to porphyria, and also in cases of dermatitis of phototoxic origin: drug-induced photosensitization, sun-induced urticaria, or lupus erythematosus summer flares. These products ( $\beta$ -carotene, canthaxanthin, in combination) are used orally; they are contraindicated in case of retinal disease or glaucoma. The risk that canthaxanthin will crystallize in the retina requires close medical supervision of the vision when the treatment exceeds two months per year. Contact lens wearers must be aware of the possibility of color modifications to vision.

- Carotenes are ingredients of tanning pills: in sufficient doses, they color the skin where they are deposited (but they do not provide any protection from UV radiation).

- In the pharmaceutical as well as food technology industry, carotenoids have applications as natural, efficacious, and non-toxic colorings. Currently, the following are used as food colorings: either natural extracts (annatto extract, Eur. id. code E160b, paprika E160c), or carotenes extracted from natural sources (carrots [carotene], tomato skin [lycopene]), *Tagetes erecta*, alfalfa [xanthophylls]), or synthetic carotenoids ( $\beta$ -carotene E160a, lycopene E160d,  $\beta$ -apo-8'-carotenal E160e, and xanthophylls E161, for example canthaxanthin E161g).

The natural products are commercially available as extracts.

Pure carotenoids are available in two forms: as a microcrystalline suspension in a vegetable oil or powders to be dispersed in water (dispersed in a polysaccharide-type phase or atomized oil/water emulsion). The chief applications, outside of the

\* Some authors have pointed out that this does not represent prevention, since  $\beta$ -carotene supplementation was introduced well after the smoking had started. In animals, it has been shown that carotenoids can act against each of the phases of cancer development.

pharmaceutical and cosmetic industry, are in the food industry: cold meat products and casings, dairy products, seasonings, soups, candy, pastries, liquors, beverages, and syrups, among others. Products such as paprika extract have no acceptable daily intake, but the majority of carotenes have one at 5 mg/kg. The animal feed industry also uses carotenoids (for example, to enhance the pigmentation of poultry meat and the color of eggs).

#### 4. CHIEF CAROTENOID-CONTAINING PLANTS USED TO OBTAIN COLORING EXTRACTS

- **CAPSICUM**,  
*Capsicum* spp., Solanaceae

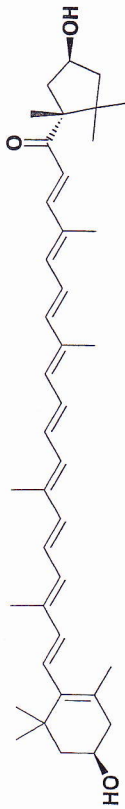
The fruits of various species and cultivars of the genus *Capsicum* (bell peppers, paprika, Cayenne pepper, chili peppers) are widely consumed for food preparation, especially to season bland foods (rice, corn, cassava); they are also used as spices, alone, or in mixtures. In food technology, paprika and its oleoresin are used as colors and flavors.

We shall not concern ourselves with distinguishing the various species and varieties: as mentioned in the French standard regarding "so-called extra hot peppers and whole or powdered hot peppers" (NF V 32-065 [1985]), "the dimensions, shape, and color vary greatly and no useful classification is possible using traditional criteria". The standard specifies that it covers two genera, *C. annuum* L. and *C. frutescens* L., as well as their hybrids, *C. chinense*, *C. pubescens*, and *C. pendulum*; it also emphasizes that the species of extra hot peppers "all contain a pungent principle consisting chiefly of capsaicinoids". It describes criteria to differentiate hot peppers and extra hot peppers, and proposes a non-exhaustive list of hot and extra hot species. The bell pepper and paprika are also standardized (NF 32-140 [1975], 32-166-167 and 32-176 [1989]).

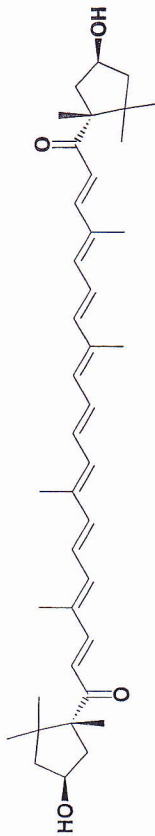
**Chemical Composition.** Capsicums are rich in ascorbic acid; they also contain diterpenoid glycosides (capsianosides) and a furostanol glycoside (capsicoside). Their color is due to the presence of carotenoids with a terminal cyclopentane ring, the level of which increases during ripening: among them capsanthin—a 3,3'-dihydroxy- $\beta$ , $\kappa$ -caroten-6'-one—occurs alongside capsorubin (3,3'-dihydroxy- $\kappa$ , $\kappa$ -caroten-6,6'-dione), capsanthinone, cryptocapsin, violaxanthin, and  $\alpha$ -carotene. The pungent flavor of hot and extra hot peppers is due to amides found in a wide range of concentrations, namely the capsaicinoids. The chief constituent in this series is capsaicin, which is the vanillamide of 8-methylnon-6-enoic acid. The concentration of capsaicinoids is very low in bell peppers, but can exceed 1% in the very pungent species. The quantitation of capsaicinoids is achieved by HPLC (standard NF V 32-070).



CAPSICUM ANNUM L.



Capsanthin



Capsorubin

The potential therapeutic applications of capsaicin are a subject of active research, particularly in the context of exploring the functions of certain types of neurons: it induces the selective degeneration of the primary sensory efferent amyelinated neurons; in addition, capsaicin induces the release of substance P and inhibits its reuptake, which causes its complete depletion. Capsaicin application on the skin causes a burning sensation which turns into anesthesia by inhibition of neurotransmission by C fibers (unmyelinated, of smaller diameter than A fibers, transmit impulses for non-discriminatory sensitivity). It does not affect the sensitivity to touch or heat. It does not alter the sensitivity of A fibers, which are responsible for finer discrimination.

In some countries, capsaicin-based magistral preparations are used; in other countries, proprietary products are available. For example, in the United States, lotions and creams containing 0.025-0.25% capsaicin are available. Such formulas have been tested to treat various algias of neurological origin: painful diabetic neuropathy, painful neuropathy linked to the trigeminal nerve, the pain following shingles (herpes zoster). For the latter indication, the published clinical trial results unambiguously demonstrate efficacy. Although some authors point out that the initial burning sensation makes it difficult to keep the subject blind, organizations such as the FDA have approved the use of capsaicin for this indication by topical application. Capsaicin has also been undergoing clinical tests in urology. In California, *Capsicum* oleoresin is an ingredient of pepper sprays used for self-defense (according to Dasgupta and Fowler, see bibliography).

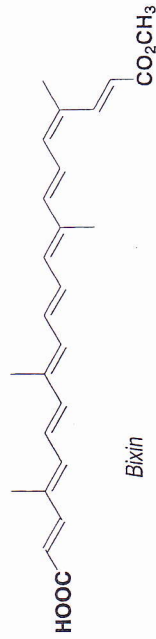
Topically, *Capsicum* is traditionally used for the symptomatic treatment of minor pain in the joints.

- **ANNATTO TREE**,  
*Bixa orellana* L., Bixaceae

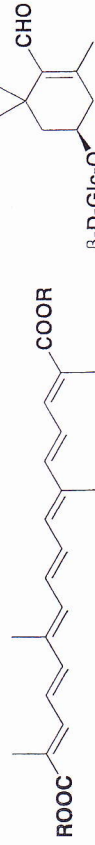
The annatto tree grows wild in the tropical areas of America, has been naturalized in all other tropical areas (India, eastern Africa), and is widely cultivated in Brazil and Peru. Some authors consider it the sole species in the sole genus in the family, while others include the genera *Cochlospermum* and *Amorpheia*. The fruit is an ovary

capsule of about three centimeters in diameter, which contains 15-20 seeds embedded in a gooey bright red pulp. The coloring known as *annatto* or *roucou* was formerly used for body painting in the Caribbean Islands. The coloring is concentrated in the seed envelope, of which it represents up to 4% in selected cultivated trees. The main constituent is 9'-cis-bixin, which is the lipid-soluble methyl ester of a C<sub>24</sub> dicarboxylic acid, 9'-cis-norbixin; cis-bixin occurs alongside small quantities of trans-bixin and cis-norbixin. The composition of the extracts depends on the extraction procedure (e.g., cis-trans isomerization upon heating, hydrolysis in alkaline conditions).

The crude product is used to prepare oily extracts containing 0.2-0.3% bixin, soluble extracts containing 0.5-4% norbixin (sodium or potassium salts of norbixin produced by alkaline hydrolysis), or sprays with a gum, maltodextrin, or modified starch as a base (the norbixin content can then reach 14%). Synthetic bixin is also available. The extracts are relatively stable to heat and light, but they are sensitive to oxygen. Norbixin is acidic, therefore it cannot be used in acidic conditions because it would precipitate. The extracts are mostly used as food coloring, for example in dairy products (Eur. id. code E160b, acceptable daily intake: 2 mg/kg); they are sometimes used to color salad oils rendered too pale by deodorization procedures.



Bixin



R = H : Crocetin

R = β-D-Glc-(1→6)-β-D-Glc-(1→) : Crocin

Picrocrocin

- **SAFFRON**,  
*Crocus sativus* L., Iridaceae

This small plant of oriental origin grows from a bulb and is used for its stigmas. These (Fr. Ph., 10th Ed.) have an aromatic odor, and a slightly bitter and pungent taste. They are elongated, filamentous, and dark red; their distal end is split longitudinally and rolled into a slender funnel with a crenate edge.

The color of the drug is due to carotenoids, chiefly represented by crocin (2%), which is the diester of crocetin (8,8'-diapocartene-8,8'-dioic acid) and of gentiobiose. Other constituents include picrocrocin (4%), which is the glucoside of 4β-hydroxycyclocitral, a small amount (1%) of essential oil in which safranal

hydrolysis of picrocrocin), phenylethanol, and trimethylated cyclohexane-type derivatives.

Because the high price of the drug leads to illicit attempts at substitution or addition, the assay is crucial: it includes examining the macroscopical characteristics; verifying the absence of turmeric (pear-shaped starch grains), capsicum (sclerified cells), marigold or safflower (pollen grains with three pores); foreign matter (<2%); chromatographic analysis of an aqueous extract; characterization of crocin (color in the presence of sulfuric acid); recording the absorbance spectrum between 240 and 450 nm and measuring the bands due to picrocrocin, safranal, and crocin. (See also, on the topic of quality control, French standard NF V 32-120, 1 and 2, 1991).

Saffron stigmas are seldom used in pharmacy: officially, preparations based on the stigmas may be used topically "to relieve teething pains in children" [French Expl. Note, 1998]. The tincture is also used as a coloring. The German Commission E monograph describes that saffron stigma is a traditional nervous sedative, but that there is no proof of its activity; therefore, its therapeutic use is not recommended. At high doses, (i.e., >5 g), the drug is thought to be toxic. In fact, the dried stigmas are primarily used as a spice (whole filaments, cut filaments, powder).

#### ● PALM OIL as coloring

The addition of coloring to margarine is still prohibited in France, but what is allowed is the use of naturally colored fats in the manufacturing process! To color the oily phase of margarine, palm oils titrated for their color are commonly used: they contain, after being refined and deodorized, between 300 and 500 ppm of total carotenoids.

Palm oil can also be a good source of carotenes. The oil is transesterified by methanol, which allows the separation of the esters (for the detergent industry) and a fraction enriched in carotenes. These (60-70%  $\beta$ , 30-35%  $\alpha$ ) are then purified by chromatography.

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