

# Carotenoid Biosynthesis — a Target for Herbicide Activity

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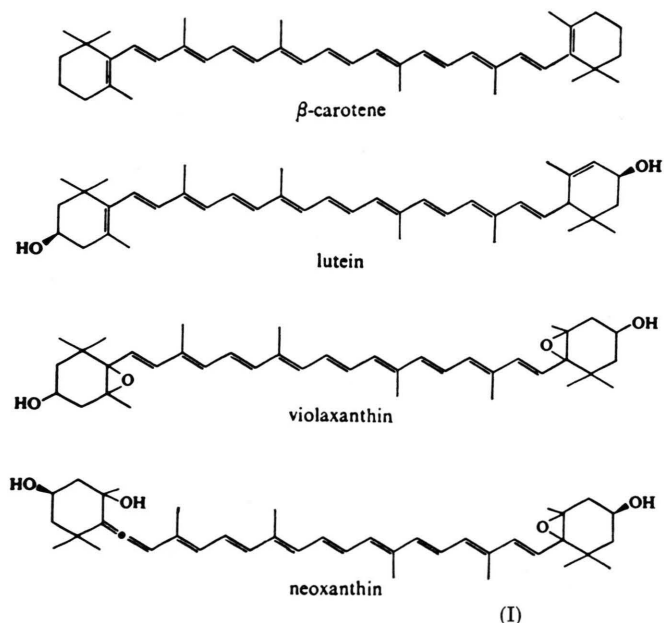
Carotenoids are chloroplast constituents. *In vivo* different carotenoids may be present at different sites within the chloroplast, and may have different functions. Especially important is a role in protecting the chloroplast and hence the plant from chlorophyll-sensitized photooxidation. If carotenoid biosynthesis can be blocked by herbicides, such protection will no longer be afforded, and rapid death of the plant is likely. In this article current knowledge of carotenoid biosynthesis is reviewed. The main characteristics of each stage in the biosynthesis are outlined and the suitability of these reactions as possible herbicide targets is assessed. Two experimental approaches to the study of the effects of herbicides on carotenoid biosynthesis are suggested.

## Introduction

Green tissues of all higher plants contain the same major carotenoids,  $\beta$ -carotene, lutein, violaxanthin, and neoxanthin. Smaller amounts of  $\alpha$ -carotene,  $\beta$ -cryptoxanthin, zeaxanthin, and antheraxanthin are commonly also present. All these pigments are located in the chloroplasts, primarily in the light-harvesting antenna complexes of photosystems I and II, [1], but also perhaps in the chloroplast envelope [2]. There is evidence of differences in the carotenoid compositions of different sub-chloroplast fractions or particles, for example photosystem I is reported to be enriched in  $\beta$ -carotene and photosystem II in lutein [3], whereas zeaxanthin and violaxanthin have been obtained from chloroplast envelope preparations [2]. The carotenoids at the various sites are certainly in different microenvironments, and are likely to be maintained in specific spatial orientations or as specific molecular complexes with protein and lipid.

The chloroplast carotenoids function as accessory light-harvesting pigments [1] and it has been suggested that some special carotenoid molecules may be involved in photosynthetic electron transfer [4]. In the context of herbicide activity the protective function of carotenoids against photooxidation is probably of greatest importance [5–7]. At high light intensities the chlorophyll of the light-harvesting antennae is capable of absorbing more light energy than can be passed on to the photosynthetic

reaction centres and thus into the electron transfer system, and a large proportion of the chlorophyll molecules become electronically excited. Various pathways exist for the dissipation of this excitation energy including intersystem crossing to form a triplet state,  $^3\text{CHL}$ . This longer lived  $^3\text{CHL}$  can return to the ground state by passing on energy to the ubiquitous molecular oxygen,  $^3\text{O}_2$ , thus producing singlet oxygen,  $^1\text{O}_2$ . This powerful oxidizing species is highly destructive and can rapidly oxidize chlorophyll and the photosynthetic apparatus. The importance of carotenoid lies in providing a means of preventing this oxidative damage by  $^1\text{O}_2$ . Caro-



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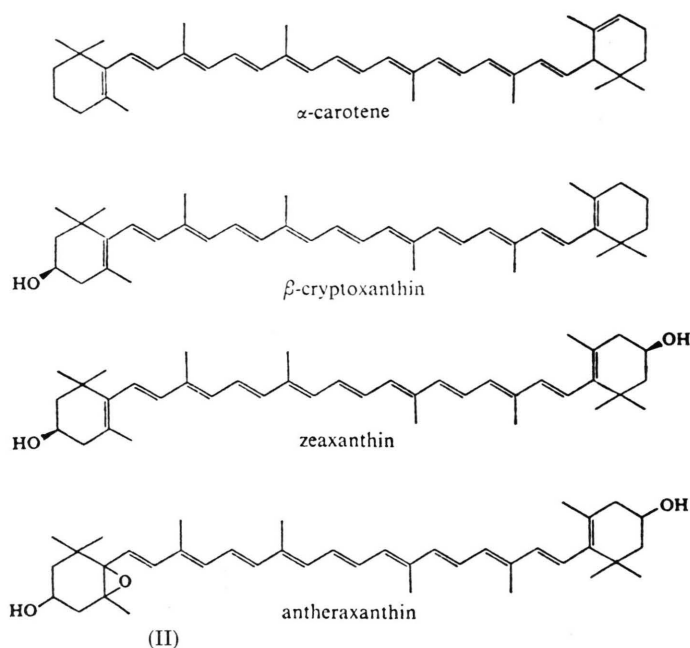


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tenoid can quench efficiently the excess energy of  $^3\text{CHL}$ , thus preventing  $^1\text{O}_2$  formation, or can rapidly quench the excess energy of  $^1\text{O}_2$  if this is produced.

The triplet carotenoid produced loses its excess energy harmlessly as heat. Photooxidative damage is therefore prevented. However if carotenoid is for some reason not present then no such protection is available and chlorophyll and the photosynthetic apparatus will rapidly be destroyed. The biosynthesis of carotenoids should therefore be a prime target for herbicide activity.

### Carotenoid biosynthesis

Space permits only a brief outline of carotenoid biosynthesis. Some aspects that may be especially relevant to herbicide action will be pinpointed. Further details are available in a number of recent reviews [8–15].

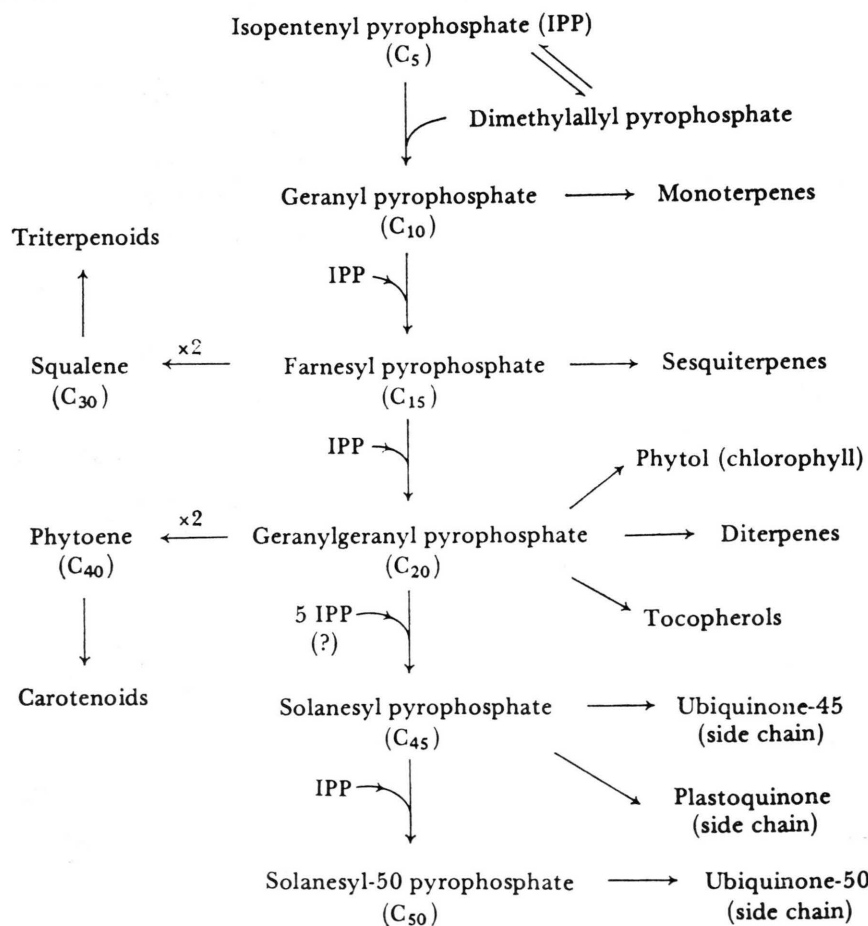


Fig. 1. General pathway of isoprenoid biosynthesis in higher plants

### The general isoprenoid pathway

Carotenoids are terpenoids, biosynthesized by the general isoprenoid pathway from acetate and mevalonate. This pathway (Fig. 1) also gives rise to various mono-, sesqui-, and diterpenes, sterols, isoprenoid quinones and the phytol sidechain of chlorophyll. The early stages of the pathway, up to farnesyl and geranylgeranyl pyrophosphates, are common to the biosynthesis of all isoprenoid compounds. Although isoenzymes may well be involved, any substances that affect the early stages of carotenoid biosynthesis would presumably also affect the formation of other isoprenoids, particularly those outside the chloroplast, since the enzymes of their formation would be more accessible.

### The formation of phytoene

The first process specific to carotenoid biosynthesis is the formation of phytoene, the first C<sub>40</sub> carotene intermediate from geranylgeranyl pyrophosphate. In plants the phytoene produced appears to be the 15-*cis* isomer, though *trans*-phytoene is formed directly in some bacteria. An intermediate, prephytoene pyrophosphate, is involved, and possible mechanisms have been suggested [16].

Enzyme systems capable of phytoene biosynthesis have been isolated from bacteria [16–18], fungi [12, 16] tomato fruit plastids [8, 19, 20] and chloroplasts [21]. No coenzymes are involved in the reaction but in the tomato system phytoene production seems to be enhanced by the presence of ATP [20].

Phytoene synthesis would probably be a difficult target for herbicides, since the reaction mechanism is similar to that of the formation of squalene, the sterol precursor. Squalene and sterol biosynthesis occur outside the chloroplast and would therefore be affected preferentially. The phytoene synthetase enzyme system is, however, a likely control point; this enzyme is known to be photoinduced in some bacteria.

### Desaturation

Phytoene has only a short chromophore of three conjugated double bonds and is therefore colourless ( $\lambda_{\text{max}}$  275, 285, 296 nm). Its conversion into the normal coloured carotenoids requires a series of desaturation reactions. In each of these, two hydrogen atoms are removed in a *trans* elimination to in-

troduce a further double bond and extend the chromophore by two conjugated double bonds (Fig. 2). In higher plants these desaturations occur alternately in the two halves of the molecule to give successively phytofluene,  $\zeta$ -carotene, neurosporene, and finally lycopene. Since in higher plants the phytoene appears to be the 15-*cis* isomer whereas the coloured carotenoids are all-*trans*, an isomerization must occur at some stage during the desaturation process. This isomerization may occur at the phytoene, phytofluene or  $\zeta$ -carotene level in different systems.

The carotenoid desaturation reactions have been demonstrated with crude cell-free systems from tomato plastids, [8, 22–24] fungi [12] and bacteria [18, 25], but there is conflicting evidence about the requirement for the coenzymes NAD(P)<sup>+</sup>, NAD(P)H, FAD(H<sub>2</sub>) and FMN(H<sub>2</sub>).

Investigation of the desaturation enzyme system is very difficult since it appears that a great deal of structural integrity and organization must be retained in the cell or plastid membrane system if the ability to bring about desaturation is not to be lost. A multi-enzyme complex which may be an integral part of the membrane structure is therefore suggested, a concept which has received support from genetic studies with carotenogenic fungi [26]. The desaturation reactions are oxidative, and there is some evidence that metal ions or cytochromes in the form of a simple electron transfer system may be intimately associated with the desaturation enzyme complex [25].

Carotenoid desaturation is relatively easy to inhibit. Mutant strains of bacteria, fungi, algae and higher plants have been obtained which are unable to synthesize the normal coloured carotenoids but accumulate phytoene. Similarly a number of chemicals have a similar effect, notably diphenylamine, an inhibitor widely used in classical studies of carotenoid biosynthesis, and the pyridazinone herbicides, Sandoz 6706 and 9789 [27, 28].

The desaturation sequence should be a good target for herbicide action. It is specific to carotenoid biosynthesis and seems to be relatively easy to block. Also, if desaturation is inhibited, an accumulation of phytoene results. Phytoene is useless to the chloroplast; its short chromophore cannot afford protection against photooxidation, so plants in which desaturation is blocked will rapidly be killed by light and oxygen.

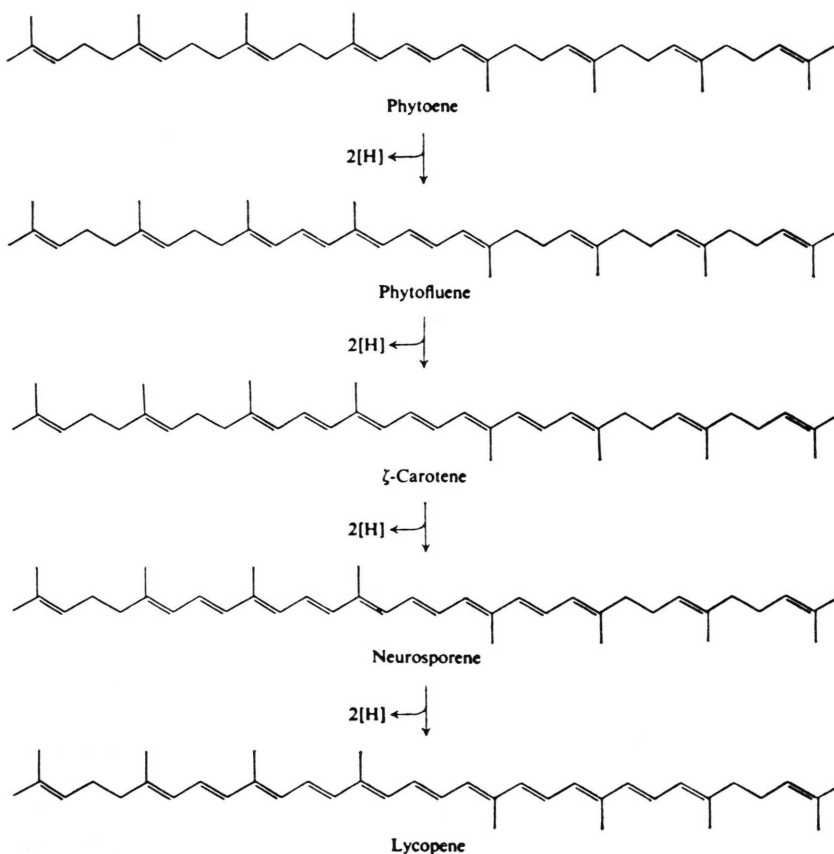


Fig. 2. The desaturation sequence from phytoene to lycopene.

Unfortunately not enough is known about the desaturation enzyme system to allow predictions of molecular structures which may be likely inhibitors or to explain the inhibitory action of compounds such as diphenylamine and the Sandoz herbicides. Desaturation is an oxidative reaction and these compounds may simply be inhibitors of oxidative processes, not specifically of carotenoid biosynthesis. The effects on carotenoid biosynthesis are immediately obvious, because of the colour changes produced, but other similar reactions could also be affected. The inhibition of fatty acid desaturation by pyridazinone herbicides has been reported [29].

### Cyclization

The normal chloroplast carotenoids have cyclic end-groups, in the form of  $\beta$ - and  $\epsilon$ -rings. The likely mechanism for the cyclization of an acyclic precursor to give these end-groups is illustrated in Fig. 3. There has been considerable argument over whether it is neurosporene or lycopene that cyclizes, and the

possible alternative routes for  $\beta$ -carotene formation are shown in Fig. 4. A similar scheme could be given for  $\alpha$ -carotene.

The cyclization of acyclic carotenoids, particularly lycopene, has been demonstrated with cell-free systems from tomato plastids [22, 24, 30, 31] chloroplasts [31], fungi [12] and bacteria [17, 25]. The proton attack mechanism has been confirmed [32] and the stereochemical course of  $\beta$ -ring

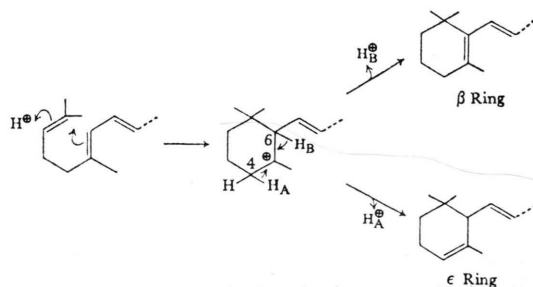
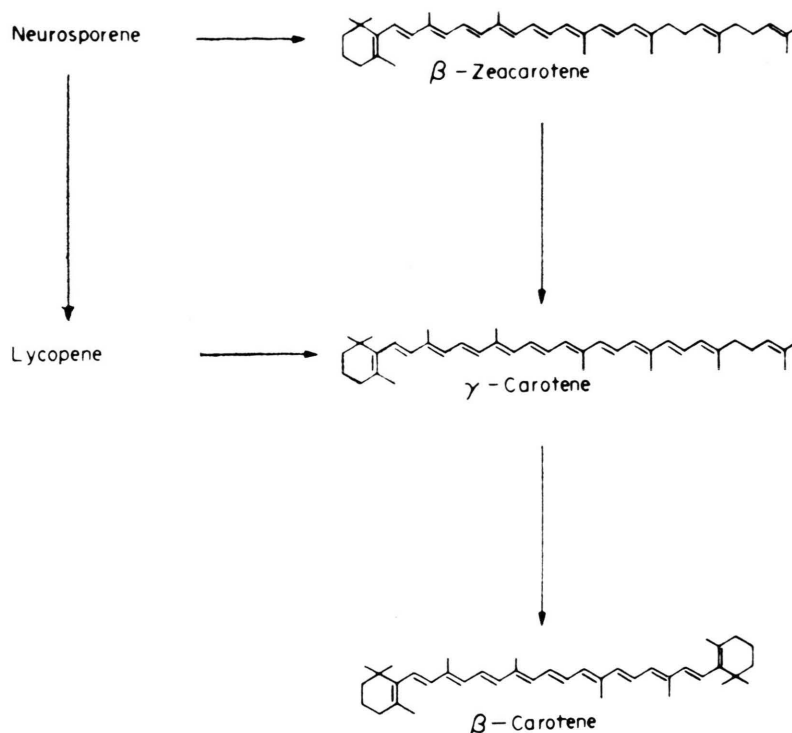


Fig. 3. A mechanism for the formation of carotenoid  $\beta$ - and  $\epsilon$ -rings.

Fig. 4. Alternative routes for  $\beta$ -carotene biosynthesis from neurosporene and lycopene.



formation has been established in a bacterium [33, 34]. Apparently no cofactors are required for the cyclization.

Carotenoid cyclization is susceptible to chemical inhibition; nicotine and CPTA[2-(4-chlorophenylthio)triethyl ammonium chloride] have been used extensively as inhibitors [14]. These compounds are fairly specific but their mechanism of action is not known. The inhibition causes lycopene to accumulate in place of the normal cyclic carotenoids. The long chromophore of lycopene can afford efficient protection against photooxidative damage, but lycopene cannot fit into the sub-chloroplast structures in the same way as  $\beta$ -carotene and more especially xanthophylls, so chloroplast structural development and photosynthetic capability will be restricted. The action of herbicides which block carotenoid cyclization specifically would be expected to be slower and less drastic than the effect of desaturation inhibitors. As with desaturation, insufficient is known about the cyclase enzymes to permit any explanation of the mechanism of inhibition.

#### Introduction of oxygen functions

The introduction of oxygen functions of xanthophylls is believed to be the final step in the bio-

synthesis, *e.g.* zeaxanthin is formed from  $\beta$ -carotene, lutein from  $\alpha$ -carotene. Mechanisms have also been proposed for the conversion of zeaxanthin into violaxanthin and neoxanthin [35].

No good cell-free systems are available for studying xanthophyll biosynthesis. A crude preparation from a *Flavobacterium* species which synthesizes large amounts of zeaxanthin seems to be the most promising [25]. Some information has been obtained about hydroxylation at C-3 of the carotene ring [36], which apparently occurs by a mixed-function oxidase reaction, requiring molecular  $O_2$ , cytochrome P 450 and a reducing cofactor such as NADPH.

No substances are known to inhibit specifically this hydroxylation reaction. If this could be done, carotenes would accumulate in place of the normal xanthophylls. The carotenes would be effective in protecting against photooxidation but detrimental effects on chloroplast structure and photosynthetic efficiency would be expected. It is likely that a substance inhibiting carotenoid hydroxylation would also affect other oxidative processes such as carotenoid desaturation, formation of squalene epoxide and sterols, so that no specific effect on xanthophyll formation would be seen.

### *Deposition of carotenoids into specific sub-chloroplast structures*

As well as the actual pathways and reactions of carotenoid biosynthesis it is necessary also to consider how the carotenoids, once made, are complexed with protein or incorporated into specific sites in the photosynthetic apparatus. These processes could also be susceptible to chemical interference, in which case the carotenoid would not be available to perform its normal function, and detrimental effects on the health of the plant would be observed.

### Conclusions

The inhibition of carotenoid biosynthesis is a likely target for herbicide activity, since carotenoids are essential to the correct construction and functioning of the photosynthetic apparatus of plants, and especially because in the absence of coloured carotenoids photooxidation can rapidly cause the death of the plant. Space has permitted only a brief survey of carotenoid biosynthesis, but those reactions which may be most susceptible to chemical interference have been pointed out, especially desaturation.

Two experimental approaches may be particularly useful for studies of carotenoid biosynthesis as a target for herbicide action. The first is simply to test the effects of substances on the carotenoid contents and compositions of plants, plant tissues or chloroplasts. Such quantitative analysis can now be performed rapidly and accurately on small amounts of material, thanks to the development of high performance liquid chromatography (HPLC) methods. For a complete picture it is essential also to determine the effects on different carotenoid pools, *e.g.* on the carotenoid compositions of the different photosystems, light-harvesting antenna complexes,

and perhaps reaction centres and of the chloroplast envelope, and even specific carotenoid-protein complexes. HPLC now makes this sort of analysis feasible, although more detailed knowledge is first required about the carotenoid compositions of these various fragments in normal chloroplasts.

It must, however, be remembered that substances which affect the carotenoid compositions of chloroplasts or sub-chloroplast fractions are not necessarily acting specifically as inhibitors of carotenoid biosynthesis. There may be many other effects, with the effect on pigmentation being seen because of obvious colour changes. Ideally enzyme systems should be used to characterize specific effects of compounds on carotenoid biosynthesis. If the mechanisms of inhibition can be understood, then the design of herbicides to block carotenoid biosynthesis should become much easier.

Unfortunately, although some preliminary studies have been reported [8, 21, 31] no good carotenogenic enzyme system from chloroplasts has yet been developed. The best cell-free systems yet available for studies of carotenoid biosynthesis are ones derived from tomato chromoplasts [8, 19, 22–24, 30], the fungus *Phycomyces blakesleeanus* [12] and a *Flavobacterium* species [17, 18, 25]. Of these, that from *Flavobacterium* appears to require a much greater degree of structural organization in the enzyme complex. It is therefore much more difficult to work with but is perhaps more similar to the chloroplast enzyme system *in vivo* than are the other two. Until a suitable enzyme preparation is obtained from chloroplasts, one of these model systems would have to be used in any attempt to investigate mechanisms of biocide action against carotenoid biosynthesis. Much more work is needed to characterize the enzymes before this approach becomes practicable.

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