

# THE BIOCHEMISTRY OF THE CAROTENOIDS

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Volume 1 Plants

# THE BIOCHEMISTRY OF THE CAROTENOIDS

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Volume I Plants

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## Volume 1

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# PREFACE TO THE FIRST EDITION

The carotenoids are not only amongst the most widespread of the naturally occurring groups of pigments, but probably also have the most varied functions; witness their known roles in photokinetic responses of plants, in phototropic responses of fish and as vitamin A precursors in mammals and birds. Pigments with such wide distribution and such diverse functions are obviously of great interest to biological scientists with very different specializations, especially as it is unlikely that the study of the functions of carotenoids is anywhere near complete.

The primary aim of the present work is to discuss the distribution, biogenesis and function of the carotenoids throughout the plant and animal kingdoms in such a way that, because of, rather than in spite of its biochemical bias, it will be of value to workers interested in all the biological aspects of these pigments. The biochemical approach is considered the most effective because, generally speaking, most progress in the study of carotenoids in living material has been achieved using biochemical techniques, be they applied by zoologists, botanists, entomologists, microbiologists or other specialists; what is even more important is that a consideration of the present position makes it certain that further fundamental progress will also be made along biochemical lines.

Although many good accounts of the pure chemistry of the carotenoids are available, the most recent and comprehensive being Karrer and Jucker's *Carotinoide* (Birkhäuser, Basel, 1948), (now available in an English translation by E. A. Braude and published by Elsevier) sufficient descriptive chemistry has been included to make this book adequately self-contained and to allow the discussion to be followed without undue difficulty. The most up-to-date spectrographic data have also been included, because spectrophotometric techniques are of great importance in identifying carotenoids in biological systems.

The first comprehensive survey of the biochemistry of carotenoids was made in 1922 by the late L. S. Palmer (*Carotinoids and Related Pigments*, Chemical Catalog Co., New York); this was followed in 1934 by Zechmeister's *Carotinoide* (Springer, Berlin) and Lederer's *Les Caroténoides des Plantes*

(Hermann, Paris), and in 1935 by Lederer's *Les Caroténoides des Animaux* (Hermann, Paris). Since then a survey such as the present one has not appeared. In order to present a full picture, much of the pre-1934 work has been reconsidered and, as far as is known, every important contribution which has appeared since that date has been discussed. Two peripheral aspects of the subject have, however, been omitted, namely (a) the qualitative and quantitative changes which the carotenoids of plant materials undergo in storage or during processing into food and (b) the carotene (pro-vitamin A) requirements of different animal species; it was felt that the former, about which a great deal has been written, was too technological to be suitable for inclusion in the present volume, whilst the latter is more suitable for a monograph on vitamin A.

The very wide distribution of the carotenoids in Nature suggests that, in spite of the superficially diverse functions ascribed to them in different living tissues, there may be some factor or property through which all these functions will eventually be correlated; any suggestion as to the nature of this common property can perhaps come most readily from a comparative approach. Apart from critically surveying the literature this book has been constructed so as to focus attention on comparative data and their possible implications. If the comparative aspects do not always appear to have been given sufficient explicit consideration it is because essential data are still lacking; it may even be hoped that when research workers realize fully the lacunae, they will be stimulated to carry out investigations on comparative lines. If this does occur then the author will feel that the book has served one of its main purposes.

To many biochemists the word 'carotenoid' stimulates the mental response 'vitamin A precursor' and no more. There is a need, which it is hoped this book fulfils, to emphasize to all concerned, directly or indirectly, with carotenoid biochemistry that a much wider view must now be taken of these pigments and that in the course of elucidating their biogenesis, metabolism, and functions, very significant advances with wide implications for our understanding of living processes are to be expected.

My sincere thanks for considerable help during the writing of this book are due to many friends and colleagues; it should be emphasized however, that none of them can be considered in any way responsible for any peculiarities which may exist in the book. Professor R. T. Williams (St. Mary's Hospital Medical School) read and criticized the original typescript; Mr. D. A. Coult (Department of Botany, The University of Liverpool) read the section on plant carotenoids and corrected many errors of nomenclature; Dr. J. Glover (Department of Biochemistry, the University of Liverpool) devoted considerable time to correcting both the galley and the page proofs, and made many valuable suggestions. Miss B. M. Morris and Miss M. W. Boggiano between them produced an unblemished typescript from a far-from-perfect manuscript; the Staff of the Liverpool University Library (especially Miss E. Whelan)

went to considerable trouble to trace and obtain obscure journals and monographs.

My greatest debt of gratitude is, however, due to Professor R. A. Morton, F.R.S. His encouragement stimulated me to begin this book and his continued unstinting help during the writing of it has been invaluable.

Conditions in the British publishing world are today extremely difficult and the long delays in publishing Scientific Books, especially monographs, tend to make them out of date before they appear. My Publishers have been most tolerant in dealing with my attempts to reduce this delay to a minimum. It is entirely due to their wholehearted co-operation, that it has been possible eventually to include information available in this country up to the end of September 1951.

T.W.G.

# PREFACE TO THE SECOND EDITION

It is just over twenty-five years since the first edition of this book was published and, as in most fields of biochemistry, profound developments have occurred in carotenoid biochemistry in the intervening years. So great have these developments been that the original small book has developed into two large volumes. In the First Edition, complete coverage of the literature was aimed at and probably to a great extent achieved; in the present edition I hope that the main developments have been fully covered and documented but in order to keep within a reasonable size, some selectivity has been observed.

The present volume (Volume I) deals with Carotenoids in the Higher Plants and Protista, and Volume II will deal with animal carotenoids. In Volume I a slightly different pattern has been followed compared with the original book. The first chapter covers the basic chemistry and properties of carotenoids necessary to follow the detailed ensuing biochemistry. Chapter 2 is new in that the basic aspects of the biosynthesis of carotenoids are confined within it. The treatment is detailed and leads on naturally to special aspects of biosynthesis unique to certain organisms (for example the formation of  $C_{45}$  and  $C_{50}$  carotenoids in some bacteria), which are dealt with in the appropriate later chapters.

In the 1950s studies on the biological function of carotenoids, other than the role of some of them as a source of vitamin A in animals, were embryonic; by the late 1970s they are approaching adolescence and certainly deserve a chapter (Chapter 3) to themselves. Problems in this area still abound but much has recently been achieved. From Chapter 4 onwards the book follows the pattern of the first edition in treating Higher Plants, Liverworts, Mosses, Algae, Fungi, Non-Photosynthetic Bacteria and Photosynthetic Bacteria, *seriatim*. A final chapter, on the Geochemistry of Carotenoids, is of necessity short because of lack of information.

I am grateful to many people for their help in preparing this book; in particular to Miss B. T. Foulkes for typing numerous drafts from manuscripts which were frequently difficult to decipher, to Mrs S. Griffiths for the final typing and to Miss G. Ferry and Miss M. Emerson for meticulous sub-editing. Dr G. Britton read the manuscripts and the final proofs and made many

penetrating comments and suggestions. Finally I wish to make a special acknowledgement to my wife who has lived through thirty years of carotenoids: without her sustained support and encouragement during that period this book and very many other things in my life would never have been possible.

T.W.G.  
*October, 1978*

#### POSTSCRIPT

In spite of unavoidable delays in publishing this book it has been possible to include references to work published in 1979–80.

T.W.G.  
*July, 1980*

# [ I ]

## NATURE AND PROPERTIES

### 1.1 INTRODUCTION

The crystalline yellow pigment *carotene* was first isolated in 1831 by Wackenroder [1] and the yellow pigments of autumn leaves as *xanthophylls* by Berzelius [2] in 1837. The existence of these pigments in green leaves was first suggested in 1827 [3] and was fully established some thirty years later by Frémy [4] and by Stokes [5]. The next important developments did not emerge until Tswett [6], exploiting his newly discovered technique of chromatography, clearly showed that there existed a whole family of carotenoids (lipochromes) and Willstätter and Mieg [7] demonstrated that they were isoprenoid derivatives. Then began the golden era of the classical studies on the structures of carotenoids by Zechmeister, who first realized that carotenoids were polyenes, by Karrer who first recognized that many carotenes had symmetrical structures and by Kuhn who first showed that they were conjugated polyenes. Much of the early work was collected in a monograph by Zechmeister [8] and a definitive work was later published by Karrer & Jucker in 1948 [9]. Since then rapid development of spectroscopic techniques, in particular nuclear magnetic resonance (n.m.r.) and mass spectrometry (m.s.), has revolutionized structural studies in the carotenoid field, as in so many other fields of natural products, and a fitting testimony to the achievements of this era of carotenoid chemistry is the massive monograph edited by Isler [10] which appeared in 1971.

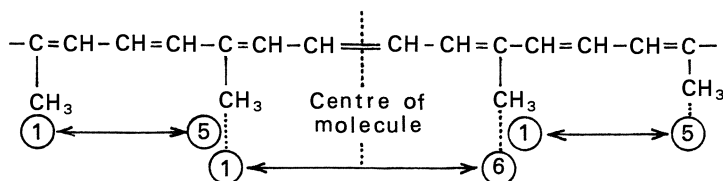
Alongside these tremendous developments in carotenoid chemistry there emerged, much more slowly, studies on the biological and biochemical aspects of carotenoids. Highlights in these early developments were covered in monographs by Kohl [11] in 1902, Palmer [12] in 1922 and Lederer [13, 14] in 1934 and 1935. The vast accumulation of chemical information, spanning some four hundred pigments, has been dealt with in great detail in Isler's book, just mentioned, and in later reviews [15–19], so it is unnecessary to deal with it again here. Indeed, the main purpose of this book is to bring up to date our knowledge of the biochemistry and biology of carotenoids

## 2 The biochemistry of the carotenoids

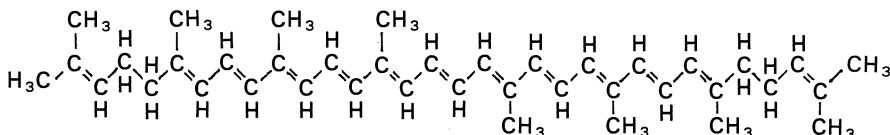
as it has developed since the first edition in 1952. Consequently, the first chapter is concerned only with basic chemical properties of carotenoids, knowledge of which is necessary for the adequate understanding of the biochemistry and biology which follow in later chapters. A good general idea of carotenoid structure is obtained by considering in outline the Rules for Carotenoid Nomenclature [19, 20].

## 1.2 NOMENCLATURE AND STRUCTURE

Carotenoids are compounds basically consisting of eight isoprenoid units (*ip*) joined so that the arrangement of the units is reversed at the centre of the molecule; thus the two central methyl groups are in a 1,6 position relative to each other whilst the remaining non-terminal methyl groups are in a 1,5 relationship (*1.1*). All carotenoids can be formally derived from the acyclic  $C_{40}H_{56}$  conjugated polyenelycopene (*1.2*) by reactions involving (a) hydro-

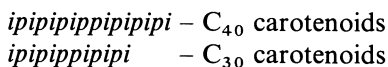


(1.1)



### (1.2) Lycopene

genation, (b) dehydrogenation, (c) cyclization, (d) insertion of oxygen in various forms, (e) double bond migration, (f) methyl migration, (g) chain elongation, (h) chain shortening. Using the abbreviation *ip* for the isoprenoid residue then the C<sub>40</sub> and C<sub>30</sub> carotenoids can be represented thus:

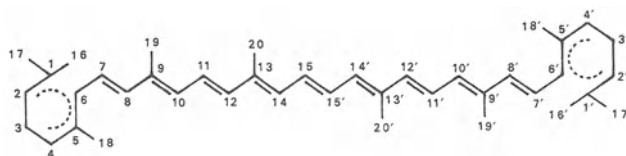


As will be seen later it is not possible to represent the C<sub>45</sub> and C<sub>50</sub> carotenoids in this simple way.

### 1.2.1 CAROTENOID HYDROCARBONS

Carotenoid hydrocarbons are known as *carotenes*. The structure and numbering of the parent carotene (stem name) is given in (1.3). Individual carotenes are named by the specific end groups which they contain. The end groups



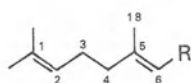


(1.3)

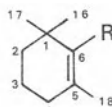
and their prefixes are indicated in Table 1.1. Examples of carotenes are

Table 1.1 End group designation of carotenes

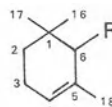
Type	Prefix	Structure
Acyclic	$\psi$ (psi)	(1.4)
Cyclohexene	$\beta$ , $\epsilon$ (beta, epsilon)	(1.5, 1.6)
Methylenecyclohexane	$\gamma$ (gamma)	(1.7)
Cyclopentene	$\kappa$ (kappa)	(1.8)
Aryl	$\phi$ , $\chi$ (phi, chi)	(1.9, 1.10)



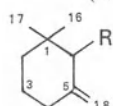
(1.4)



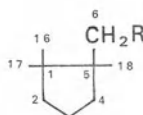
(1.5)



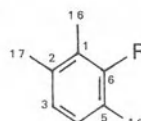
(1.6)



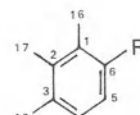
(1.7)



(1.8)

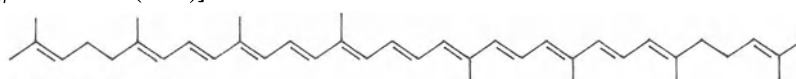


(1.9)

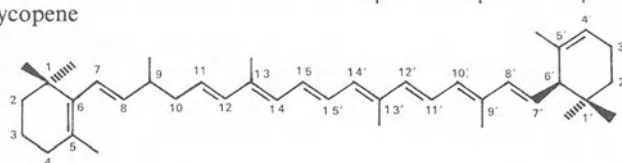
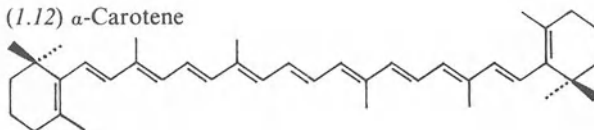


(1.10)

lycopene [ $\psi$ , $\psi$ -carotene (1.11)],  $\alpha$ -carotene [ $\beta$ , $\epsilon$ -carotene (1.12)] and  $\beta$ -carotene [ $\beta$ , $\beta$ -carotene (1.13)]. When a carotenoid is first mentioned in the text its



(1.11) Lycopene

(1.12)  $\alpha$ -Carotene(1.13)  $\beta$ -Carotene

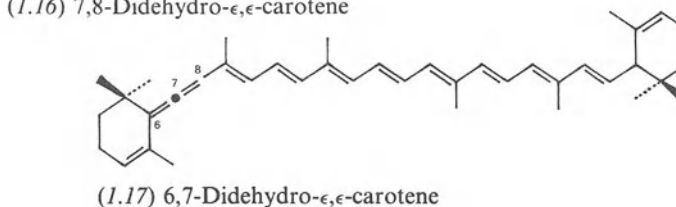
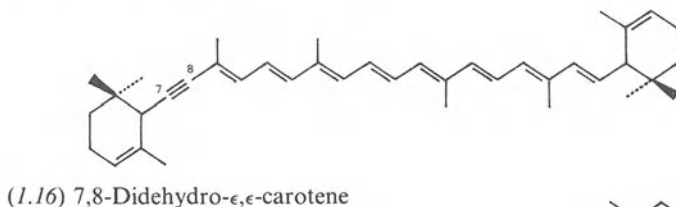
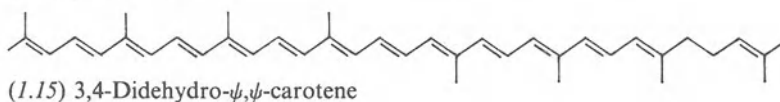
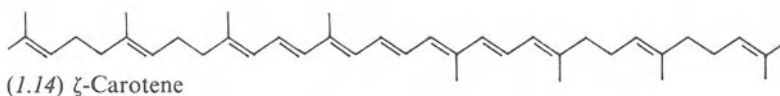
systematic or semi-systematic name will be given in brackets in addition to its trivial name, if it has one; thereafter, only its trivial name will be used.

## 4 The biochemistry of the carotenoids

For example zeaxanthin (trivial name) is  $\beta,\beta$ -carotene-3,3'-diol (systematic) or 3,3'-dihydroxy- $\beta$ -carotene (semi-systematic).

The basic system of numbering has already been illustrated in (1.3). If the carotenoid under consideration is asymmetrical, then unprimed numbers are allotted to the end of the molecule associated with the Greek letter prefix cited first in the systematic name, as indicated in the numbering of  $\alpha$ -carotene (1.12).

Changes in hydrogenation level of the parent carotenes are indicated by prefixing hydro or dehydro to the basic name, for example,  $\zeta$ -carotene [7,8,7',8'-tetrahydrolycopene (1.14)] and 3,4-didehydro- $\psi,\psi$ -carotene (1.15). Acetylenic and allenic derivatives also occur naturally; examples of such structures are given in (1.16) (7,8-didehydro- $\epsilon,\epsilon$ -carotene) and (1.17) (6,7-didehydro- $\epsilon,\epsilon$ -carotene) respectively, although it must be emphasized that almost all naturally occurring acetylenic and allenic carotenoids contain oxygen and these are considered in the next sections.



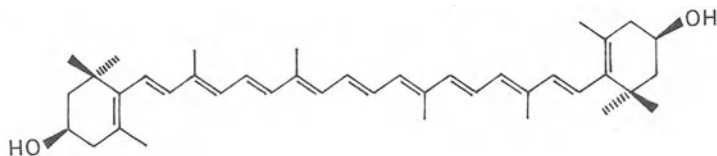
### 1.2.2 OXYGENATED CAROTENOIDS

Oxygenated carotenoids are known collectively as *xanthophylls*. These are named according to the usual rules of organic chemical nomenclature. The functions most frequently observed are (i) *hydroxy* as in zeaxanthin (1.18) ( $\beta,\beta$ -carotene-3,3'-diol); (ii) *methoxy* as in spirilloxanthin (1.19) (1,1'-dimethoxy-3,4,3',4'-tetrahydro-1,2,1',2'-tetrahydro- $\psi,\psi$ -carotene); (iii) *glycosyloxy* as in myxoxanthophyll (1.20) [2'-( $\beta$ -L-rhamnopyranosyloxy)-3',4'-didehydro-1',2'-dihydro- $\beta,\psi$ -carotene-3,1'-diol]; (iv) *carboxy* as in torularhodin

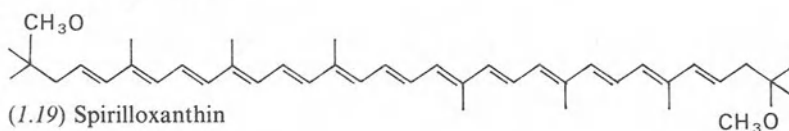
(1.21) (3',4'-didehydro- $\beta,\psi$ -caroten-16'-oic acid); *oxo* as in echinenone (1.22) ( $\beta,\beta$ -caroten-4-one); *aldehyde* as in torularhodin aldehyde (1.23) (3',4'-didehydro- $\beta,\psi$ -caroten-16'-al); *epoxy* as in antheraxanthin (1.24) (5,6-epoxy-5,6-dihydro- $\beta,\beta$ -carotene-3,3'-diol).

### 1.2.3 RETRO-CAROTENOIDS

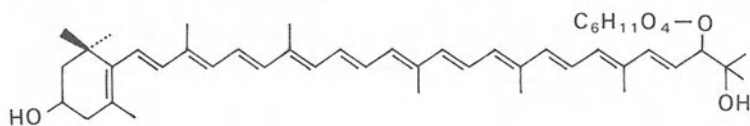
*Retro*-carotenoids are those in which all single and double bonds of the



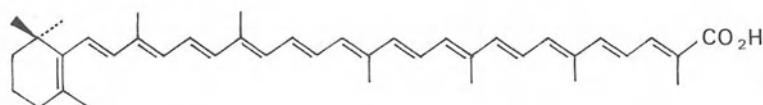
(1.18) Zeaxanthin



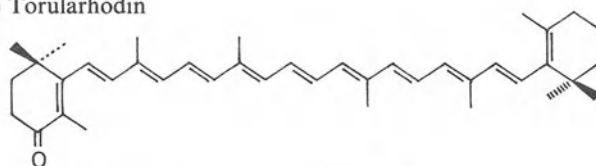
(1.19) Spirilloxanthin



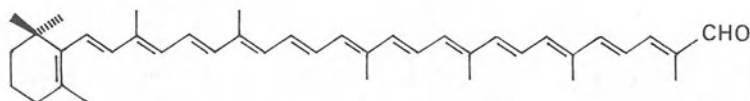
(1.20) Myxoxanthophyll



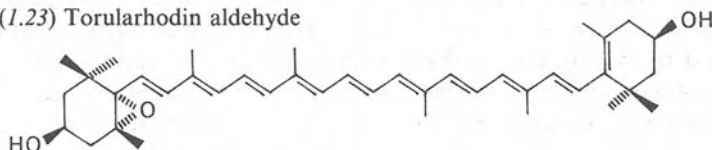
(1.21) Torularhodin



(1.22) Echinenone

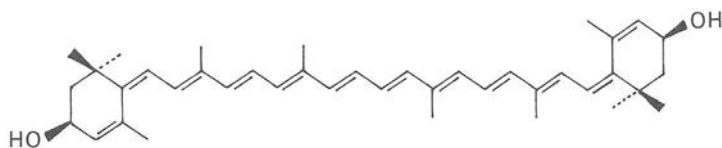


(1.23) Torularhodin aldehyde



(1.24) Antheraxanthin

## 6 The biochemistry of the carotenoids

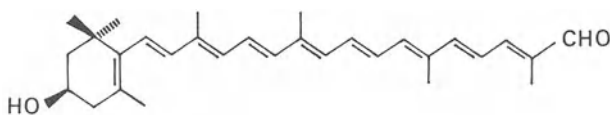


(1.25) Eschscholtzianthin

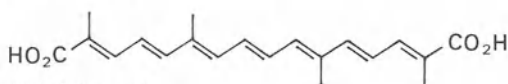
conjugated polyene system have shifted by one position. The carbon atoms delineating the conjugated systems are indicated in the systematic numbering; for example, eschscholtzianthin (1.25) is 4',5'-didehydro-4,5'-*retro*- $\beta$ , $\beta$ -carotene-3',3'-diol.

### 1.2.4 SECO- AND APOCAROTENOIDS

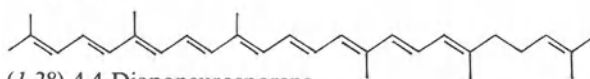
A carotenoid which formally undergoes oxidative fission without loss of any carbon atoms is called a *secocarotenoid* (see 2.23 as an example). If however the carbon skeleton has been shortened the resulting compound is called an *apocarotenoid*. The prefix apo- is preceded by the number of the carbon atom from which all the remainder of the molecule has been removed:  $\beta$ -citraurin (1.26) is thus 3-hydroxy-8'-apo- $\beta$ -caroten-8'-al and crocetin is 8,8'-diapocarotene-8,8'-dioic acid (1.27). The  $C_{30}$  carotenoids (Chapter 9) are treated as apocarotenoids for nomenclature purposes, as for example in (1.28), 4,4'-diaponeurosporene.



(1.26)  $\beta$ -Citraurin



(1.27) Crocetin



(1.28) 4,4'-Diaponeurosporene

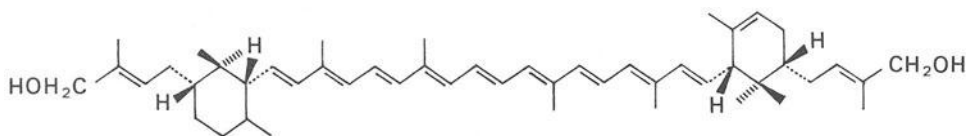
### 1.2.5 NOR-CAROTENOIDS

In nor-carotenoids, carbon atoms have been formally removed by processes other than cleavage of carbon-carbon double bonds. An important example is peridinin (1.71) (p. 26) which is 5',6'-epoxy-3,5,3'-trihydroxy-6,7-didehydro-5,6,5',6'-tetrahydro-10,11,20-trinor- $\beta$ , $\beta$ -caroten-19',11'-olide-3-acetate).

### 1.2.6 HIGHER CAROTENOIDS

Carotenoids with 45 or 50 carbon atoms are named as mono- or disubstituted

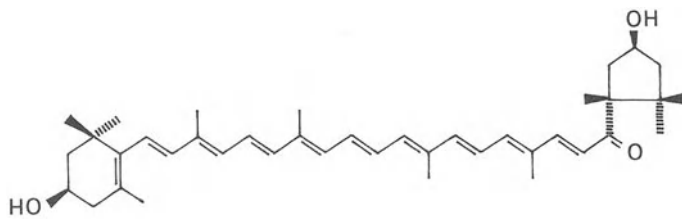
C<sub>40</sub> carotenoids; decaprenoxanthin (1.29) is thus 2,2'-bis(4-hydroxy-3-methylbut-2-enyl)- $\epsilon,\epsilon$ -carotene.



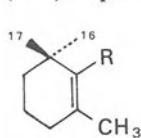
(1.29) Decaprenoxanthin

### 1.2.7 STEREOCHEMISTRY

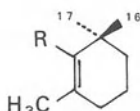
The absolute configuration at a chiral centre is designated by using the *R,S* convention\* and by placing the symbols before the name, for example capsanthin (1.30) is (3*R*,3'*S*,5'*R*)3,3'-dihydroxy- $\beta,\kappa$ -caroten-6'-one. When the potential chirality at C-1 is as in (1.31) with the polyene chain to the right of C-1 then the methyl group below the plane of the paper is numbered 16 and that above, 17: the reverse is the case when the polyene chain is to the left of C-1 (1.32). In an acyclic end group, the methyl group which is *trans*



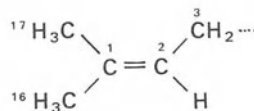
(1.30) Capsanthin



(1.31)



(1.32)



(1.33)

to the main chain is numbered 16 and that which is *cis* is numbered 17, as in (1.33).

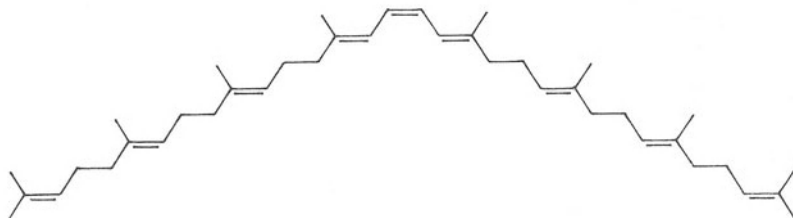
With regard to geometrical configuration around double bonds the stem name *carotene* implies a *trans* configuration at all double bonds. *Cis* configuration is indicated by citing the double bonds which possess this configuration either as *cis* or *Z*.† The *cis* isomer of phytoene which seems to predominate in nature (1.34) is thus either 15-*cis*-7,8,11,12,7',8',11',12'-

\* For a brief summary of the basic rules of the *R,S* convention see [21].

† A double bond with the highest priority groups at both ends on the same side of the axis of the double bond is designated *Z* (Zusammen). When such groups are on opposite sides of the double bond the designation is *E* (Entgegen).

## 8 The biochemistry of the carotenoids

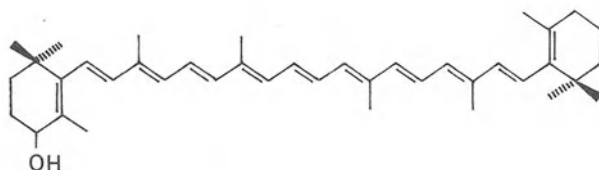
octahydro- $\psi,\psi$ -carotene or 13*E*,15*Z*,13'*E*-phytoene. Isomers with unlocated *cis* bonds are often given the prefix neo-, and a letter as a suffix, e.g. neozeaxanthin B (see also p. 21).



(1.34) 15-Cis-phytoene

### 1.3 ISOLATION AND PURIFICATION

An outline only of the general methods used will be considered because full details have been given elsewhere [22, 23]. Except when they occur as protein complexes or as glycosides, carotenoids are easily soluble in lipid solvents which are thus used for extraction. If the tissue has first been dried then water-immiscible solvents such as diethyl ether or light petroleum are used. For fresh tissues, a solvent such as ethanol or acetone will fill the double role of dehydrating agent and extracting solvent. A pigment can occasionally be crystallized directly from the extracting solvent after suitable concentration, but much more frequently a number of purification steps are necessary before this happy event occurs. If neutral lipid is abundant in the extract, saponification is first necessary followed by extraction of the carotenoids into the non-saponifiable fraction. However, some carotenoids are unstable to base, these include astaxanthin and fucoxanthin (see p. 23). Preliminary fractionation is usually achieved by column chromatography or by partition between immiscible solvents. An important hazard recently revealed is that  $\beta$ -carotene is rapidly and easily converted into isocryptoxanthin (1.35) when adsorbed on Micro-cel C [73]. Other hazards include the oxidation of



(1.35) Isocryptoxanthin

astaxanthin to astacene on alumina columns and the isomerization of 5,6-epoxides to 5,8-epoxides (see p. 25) on acid adsorbents.

Further processing of the fractions is carried out by paper or, more usually, thin-layer chromatography and eventually a product is obtained

which is crystallizable or, if only minute quantities are available, is sufficiently pure for examination by physical methods. The  $R_F$  values for most carotenoids have been recorded for paper and thin-layer systems [23]. Under closely controlled conditions, these are reproducible but preliminary identifications can only be effectively achieved by mixed chromatography with an authentic specimen of the suspected pigment in a variety of systems. Full identification requires a consideration of physical properties which are discussed in the next section. Improvement in physico-chemical techniques means that almost all physical measurements can be carried out on microgram or milligram quantities. Gas-liquid chromatography cannot be used directly for separating carotenoids because of their instability to heat. The perhydroderivatives can, however, be separated. For example, perhydrolycopene, perhydro- $\gamma$ -carotene and perhydro- $\beta$ -carotene can be separated from each other; perhydro- $\beta$ -carotene and perhydro- $\alpha$ -carotene, on the other hand, cannot be separated because they are the same compound. A list of retention times for a number of perhydrocarotenoids has been published [23]. High pressure liquid chromatography has recently been used to separate complex mixtures of carotenoids, as found in citrus fruit (see Chapter 5), and it is likely to become increasingly popular as its full potential is established.

Most carotenoids are unstable, especially to light and oxygen, and all operations with these compounds should be carried out in an inert atmosphere, in subdued light and with solvents freshly purified. Heating of solutions should be kept to the minimum. Carotenoids, either as solids or in solution in an innocuous solvent, should be stored in the dark, under nitrogen and refrigerated [22, 23].

## 1.4 PHYSICAL PROPERTIES

### 1.4.1 ABSORPTION SPECTROSCOPY

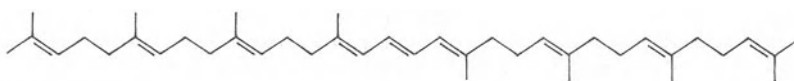
This technique has been the cornerstone for identifying carotenoids ever since it was introduced into biochemical research some fifty years ago. It is still the diagnostic tool most easily available to the majority of biochemists and biologists and it will thus be discussed at rather greater length than the other techniques considered later in the chapter.

#### (a) *Localization of absorption maxima*

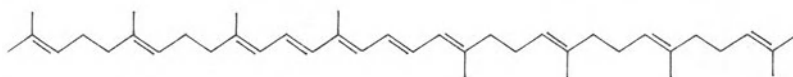
The characteristic absorption spectrum of a carotenoid is a consequence of the conjugated polyene system present in the molecule and of various additional structural features. The spectra can best be understood by reference to Tables 1.2 and 1.3. Table 1.2 summarizes the effects of various substituents on the position of the absorption maxima and Table 1.3 gives the position

## 10 The biochemistry of the carotenoids

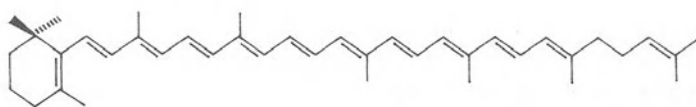
of the maxima of the carotenoids discussed in the text. The increase in wavelength of the position of the absorption maxima due to an additional conjugated double bond varies from 7 to 35 nm according to the number of conjugated double bonds already present. For example, the increase in going from *trans*-phytoene (7,8,11,12,7',8',11',12'-octahydrolycopene) (1.36) to *trans*-phytofluene (7,8,11,12,7',8'-hexahydrolycopene) (1.37), which involves the extension of the chromophore by two double bonds, is 61 nm (285 → 346 nm) (Table 1.3). The effect of inserting a double bond into conjugation in a ring is seen by comparing the main maximum of the absorption spectrum of  $\alpha$ -carotene (1.12) with that of  $\beta$ -carotene (1.13) (4nm,5). Put another way, when a  $\psi$ -end group cyclizes to a  $\beta$ -end group there is a hypsochromic shift (shift to higher frequencies, i.e. to lower wavelengths) as can be seen if the absorption maxima of lycopene (1.1) are compared with those of  $\gamma$ -carotene (1.38) (one  $\beta$ -end group) and  $\beta$ -carotene (1.13) (two  $\beta$ -end groups). The shift is due to steric hindrance between the methyl group at C-5 and the polyene chain (1.39). The resulting lack of planarity in the molecule results in a shift of about 12 nm to shorter wavelengths.



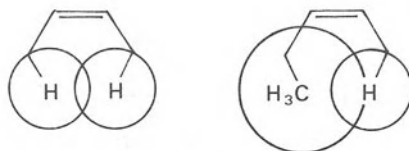
(1.36) Phytoene



(1.37) Phytofluene



(1.38)  $\gamma$ -Carotene



(1.39)

The effect of the insertion of carbonyl groups in conjugation in the ring is observed by comparing the spectra of  $\beta$ -carotene (1.13), echinenone (1.22)



Table 1.2 Effect of substituents on the main absorption maxima of carotenoids [15]

<i>Substituent</i>	<i>Position</i>	$\Delta$ nm
Double bond	chain	+7–35
Double bond	ring	+5–9
Carbonyl (First)	chain	+28
Carbonyl (Second)	chain	+1–7
Carbonyl (First)	ring	+7
Carbonyl (Second)	ring	+5–9
Epoxide 5,6	—	—8
Epoxide 5,8	—	—20
<i>Trans</i> → <i>cis</i>		—4
Normal → <i>retro</i>		—10

Table 1.3 Absorption maxima in light petroleum of some well known carotenoids. (Figures in brackets represent shoulders in the spectrum)

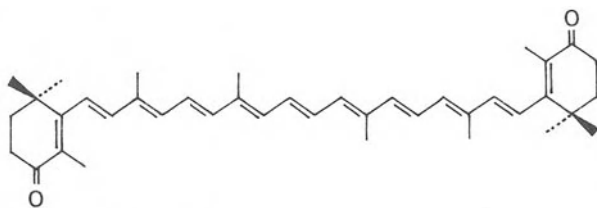
<i>Pigment</i>	<i>Absorption maxima (nm)</i>			<i>Ref.</i>
Phytoene (1.36)	276,	286,	298	24
Phytofluene (1.37)	331,	347,	367	25
$\alpha$ -carotene (1.12)	422,	444,	473	26
$\beta$ -carotene (1.13)	(427),	449,	477	15
Echinenone (1.22)		456,	(482)	27
Canthaxanthin (1.40)		467		23
Spheroidene (1.41)	429,	455,	486.5	28
Spheroidenone (1.42)	460,	482,	513	29
$\beta$ -carotene-5,6-epoxide (1.43)	423,	447,	478	30
$\beta$ -carotene-5,8-epoxide (1.44) (mutatochrome)	409,	428,	450	31
9- <i>cis</i> - $\beta$ -carotene (1.45)	(425),	443,	471	32
Zeaxanthin (1.18)	429,	451,	479	15
Eschscholtzxanthin (1.25)	444,	472,	502	33
Alloxanthin (1.46)		450,	479	34
Neoxanthin (1.47)	418,	442,	467	35
Lutein 5,6-epoxide (1.48)		443,	472	9
3,4-dehydrorhodopin (1.49)	455,	483,	517	36
Anhydrorhodovibrin (1.50)	455,	482.5,	516	36
Zeaxanthin dirhamnoside <sup>a</sup> (1.52)	428,	452,	480	37
$\gamma$ -carotene (1.38)	435,	461,	491	38
Chlorobactene (1.53)	435,	461,	491	38
Lycopene (1.11)	446,	472,	505	23
Renierapurpurin <sup>b</sup> (1.54)	464,	487,	519	39

<sup>a</sup>Recorded in acetone. <sup>b</sup>Recorded in benzene.

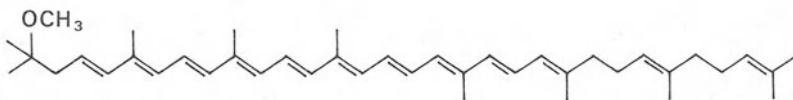
## 12 The biochemistry of the carotenoids

and canthaxanthin (1.40). Inserting a carbonyl group in conjugation with the polyene chain has a much greater effect, as can be seen by comparing the spectrum of spheroidene (1.41) with that of spheroidenone (1.42) (Table 1.3). The hypsochromic effect of insertion of oxygen across the 5,6-double bond in a  $\beta$ -ring and its rearrangement to a 5,8-epoxide is illustrated by comparing the position of the maxima of  $\beta$ -carotene with those of  $\beta$ -carotene-5,6-epoxide (1.43) and  $\beta$ -carotene-5,8-epoxide (1.44). The reactions effectively remove double bonds from conjugation so that the position and shape of the resulting spectra are very similar to those of acyclic carotenes with the same chromophore length. The effect of the *trans*  $\rightarrow$  *cis* change is observed when  $\beta$ -carotene (1.13) and 9-*cis*- $\beta$ -carotene (1.45) are compared. The normal *retro*-shift observed by comparing the wavelengths of the maxima of zeaxanthin (1.18) with those of eschscholtzxanthin (1.25) appears to be +21 nm and not -10 nm as indicated in Table 1.2. However, if one considers formally the conversion of zeaxanthin (1.18) into eschscholtzxanthin (1.25) as one in which the double bonds of the chromophore are shifted by one position, the result is a chromophore of 12 double bonds with a spectrum similar to that of lycopene (1.11) (11 double bonds). Thus the wavelength is 10nm shorter than that to be expected in an acyclic system with 12 double bonds.

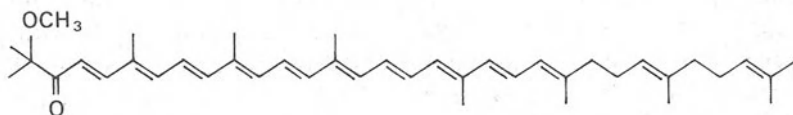
The introduction of an acetylenic linkage in place of an ethylenic bond at a hindered position has no significant effect on the spectral maxima so that the maxima of alloxanthin (7,8,7',8'-tetrahydrozeaxanthin) (1.46)



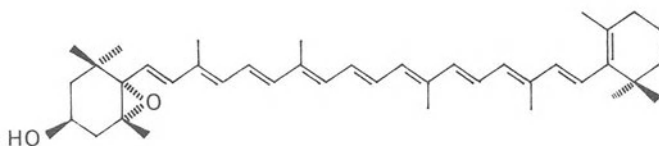
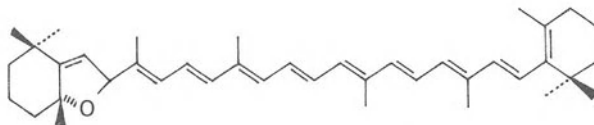
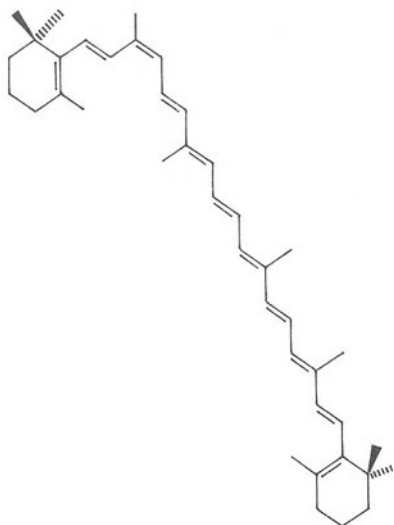
(1.40) Canthaxanthin



(1.41) Spheroidene

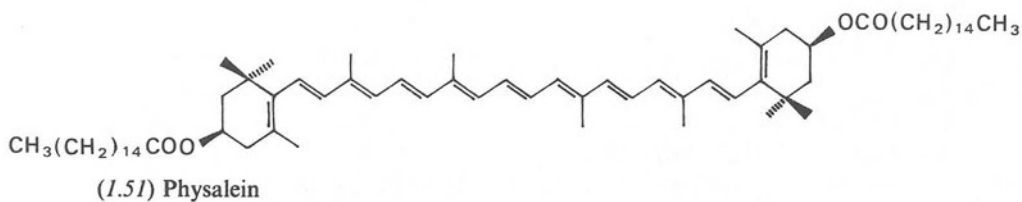
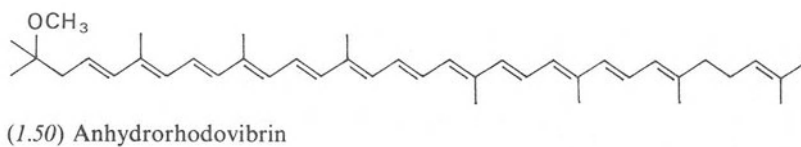
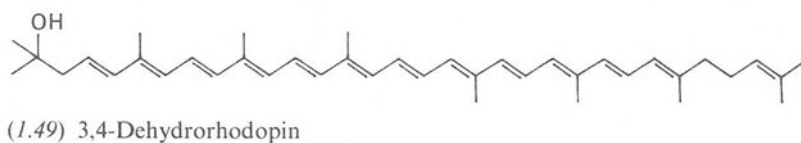
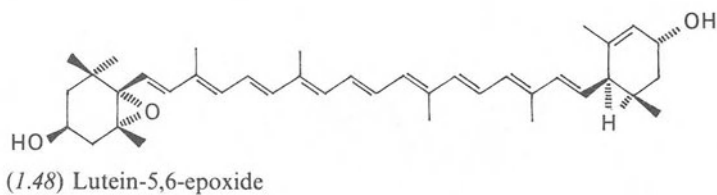
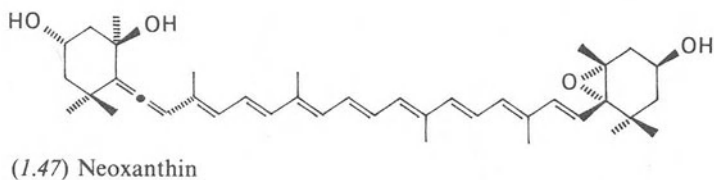
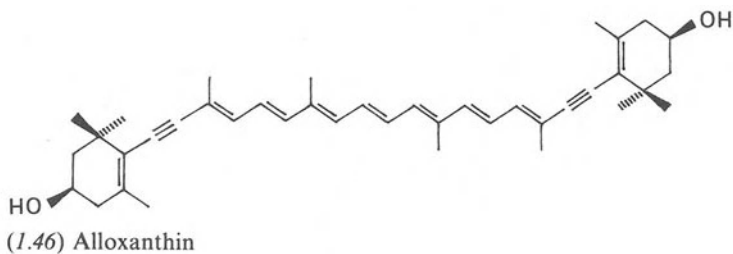


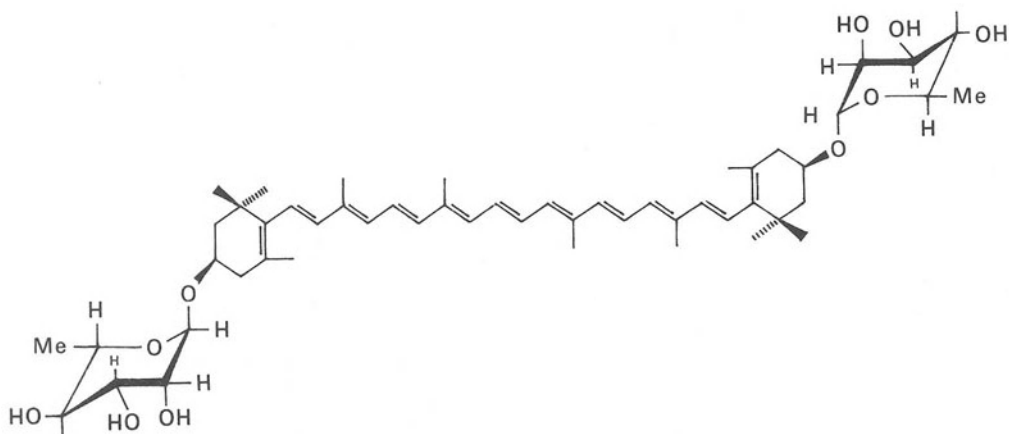
(1.42) Spheroidenone

(1.43)  $\beta$ -Carotene-5,6-epoxide(1.44)  $\beta$ -Carotene-5,8-epoxide (mutatochrome)(1.45) 9-*Cis*- $\beta$ -carotene

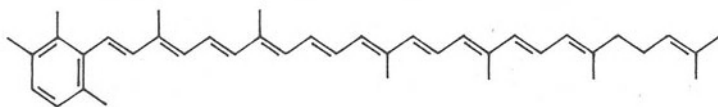
are virtually the same as those of zeaxanthin (1.18). The presence of an allene structure, as in neoxanthin [(3*S*, 5*R*, 6*R*, 3'*S*, 5'*R*, 6'*S*)-5',6'-epoxy-6,7-didehydro-5,6,5',6'-tetrahydro- $\beta,\beta$ -carotene-3,5,3'-triol] (1.47), also has no effect on the position of the maxima as can be seen by comparing it with lutein 5,6-epoxide (1.48); in both structures the chromophore ends at C-7. The insertion of a hydroxyl group into a carotenoid has very little effect on the position of the absorption maxima; compare zeaxanthin (1.18) with  $\beta$ -carotene (1.13). Neither methylation [3,4-dehydrorhodopin(1-hydroxy-3,4-didehydro-1,2-dihydrolycopene) (1.49) and anhydrorhodovibrin(1-methoxy-3,4-didehydro-1,2-dihydrolycopene) (1.50)], esterification [physalein (1.51) and zeaxanthin (1.18)], nor glycosylation [zeaxanthin (1.18) and zeaxanthin

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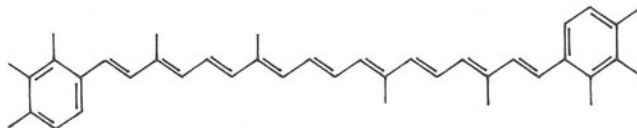




(1.52) Zeaxanthin dirhamnoside



(1.53) Chlorobactene



(1.54) Renierapurpurin

dirhamnoside (1.52)] has any marked effect. Aromatization of a  $\beta$ -ring to produce a  $\phi$ -ring has little effect on the position of the spectrum [compare chlorobactene (1.53) with  $\gamma$ -carotene (1.38)] but production of a  $\chi$ -ring, when the hindering methyl group at C-5 moves to C-3, results in the spectrum reverting to that characteristic of an acyclic [4] end group [compare renierapurpurin ( $\chi,\chi$ -carotene) (1.54) with lycopene (1.11)].

#### (b) Shape and intensity of spectra

The degree of fine structure (persistence) and the intensity of the absorption  $A_{1\text{cm}}^{1\%}$  or  $\epsilon$  (molar absorbence) also vary between types of carotenoids. The introduction of a ring double bond into conjugation with the acyclic polyene system and the conjugation of the polyene chain with another chromophore, e.g. a carbonyl group, twist the chromophore out of plane and reduce both fine structure and intensity (hypochromic effect). This is

## 16 The biochemistry of the carotenoids

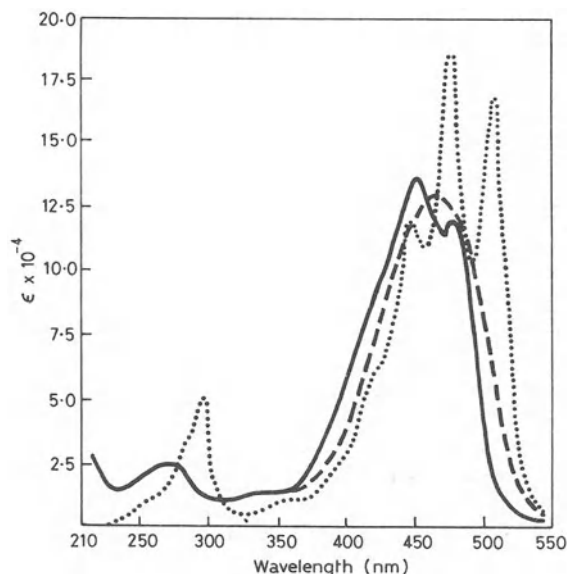


Fig. 1.1. The electronic absorption spectra of  $\beta$ -carotene (1.13) (—), lycopene (1.2) (.....) and echinenone (1.22) (-----) in light petroleum.

illustrated in Fig. 1.1 in which the spectra of lycopene (1.11),  $\beta$ -carotene (1.13) and echinenone (1.22) are compared.

The effect of geometrical isomerization on the electronic absorption spectra of carotenoids was the particular field of Zechmeister [40]. Formation of a *cis*-isomer from a parent all-*trans* compound results in a hypsochromic shift accompanied by a reduction in fine structure and a hypochromic effect on absorbance (Fig. 1.2). These changes are accompanied by the appearance of a characteristic '*cis*-peak' in the ultraviolet region of the spectrum. The position of the *cis*-peak relative to the main peak varies with the number of conjugated double bonds in the pigment; for example, for carotenoids with 11 double bonds in conjugation the *cis*-peak is located  $142 \pm 2$  nm (in hexane) lower than the peak of the longest waveband of the *trans*-compound (Fig. 1.2), whilst the value for phytofluene (five conjugated double bonds) is 108 nm. *Cis-trans* isomerization (stereomutation) can be brought about in a number of ways, including mishandling, but the most useful is by illumination in the presence of traces of iodine. A quasi-equilibrium mixture is obtained for a given compound which, with rare exceptions, is independent of which isomer is the starting material; this equilibrium mixture has a characteristic absorption spectrum which can, in some cases, be used diagnostically in attempts to identify unknown pigments. A recently discovered pigment which apparently represents an exception to this generalization is the acetylenic carotenoid alloxanthin (1.46), which on isomerization is con-

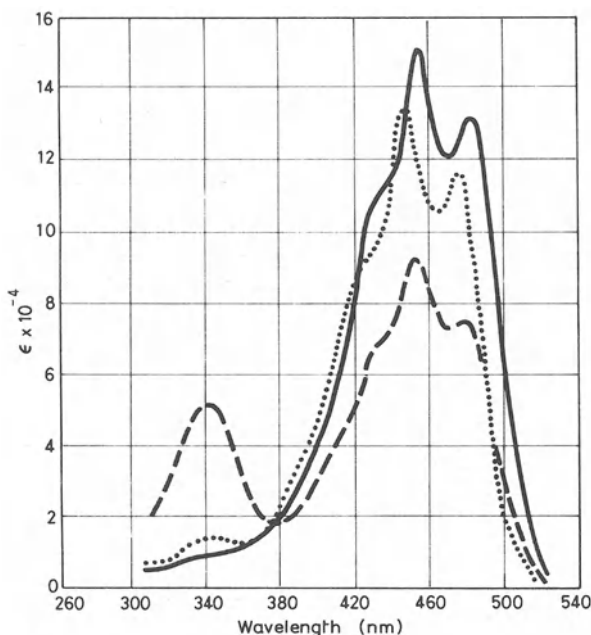


Fig. 1.2. Electronic absorption spectra of all *trans*- (——), 9-*cis* (.....) and 15-*cis*- (-----)  $\beta$ -carotene (1.13). (Redrawn from [74]).

verted almost entirely into the very stable 9,9'-*dicis*-isomer [41]. This, itself, is diagnostic.

Two groups of double bonds can be distinguished in carotenoids – *hindered* and *unhindered* [40]. The methyl substituted double bonds and the central (15,15') double bond in the  $C_{40}$  carotenoids are unhindered, whilst the remainder are hindered because of steric interference (1.39).  $I_2$ -isomerization does not, however, produce hindered *cis*-isomers although these do occur naturally and their electronic spectra are characterized by a very large hypsochromic shift, reduced fine structures and a marked hypochromic effect compared to those of the parent *trans*-compounds. On the other hand, iodine isomerization of a hindered *cis*-isomer produces the expected quasi-equilibrium mixture of isomers. Prolycopene (1.55) is an example of a carotene with hindered *cis*-double bonds and only recently has its structure been determined [42]. The dramatic change in its spectrum on  $I_2$ -isomerization is illustrated in Fig. 1.3; the effect is so marked that it is accompanied by a colour change which is visible to the naked eye.

Unhindered *cis*-isomers are also found in nature, although some of the earlier reports of their occurrence have to be reviewed cautiously because the rough treatment meted out to the extracts probably caused isomers to be formed artefactually. One well-authenticated *cis*-isomer is gazaniaxanthin

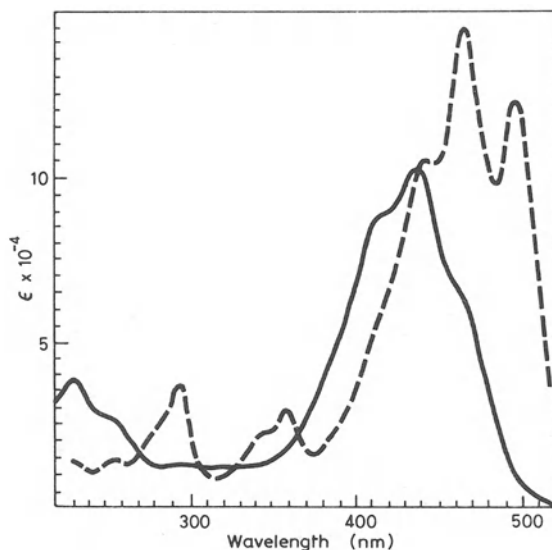
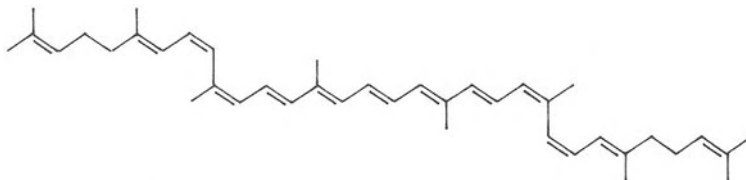
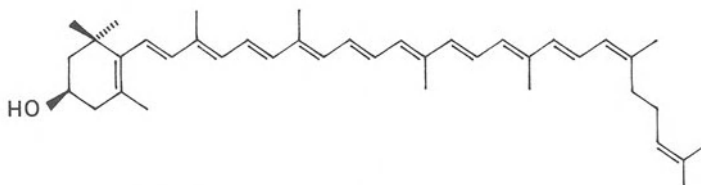


Fig. 1.3. Electronic absorption spectrum of prolycopene (1.55) in hexane: (—) fresh and (----) after  $I_2$  catalysis. (Redrawn from [74]).

(1.56) from *Gazania rigens* petals (see Chapter 5) which is 5'-*cis*-rubixanthin (3-hydroxy- $\gamma$ -carotene) [42].



(1.55) Prolycopene



(1.56) Gazaniaxanthin

### (c) Solvent effects

The solvent in which the absorption spectrum of a carotenoid is measured has a marked effect on the position of the maxima and on the molecular absorbance of the compound. These variations are indicated in Table 1.4 in which  $\beta$ -carotene is taken as a typical example.

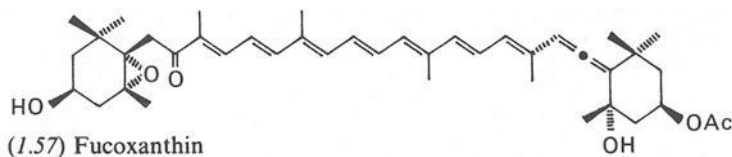


Table 1.4 Effect of solvent on absorption maxima and  $A_{1\text{ cm}}^{1\%}$  of  $\beta$ -carotene [43]

Solvent	Position of main absorption maximum (nm)	$A_{1\text{ cm}}^{1\%}$
Hexane (light petroleum)	453	2592
Ethanol	453	2620
Cyclohexane	457	2505
Benzene	465	2337
Chloroform	465	2396
Carbon disulphide	484	2008

#### 1.4.2 INFRA-RED SPECTROSCOPY (i.r.)

Infra-red spectroscopy of carotenoids has been considered in detail elsewhere [15]; here we shall only give one or two examples because this technique is not used routinely for identifying carotenoids. Infra-red spectroscopy was particularly useful in demonstrating the presence of the allene group ( $1920\text{--}1930\text{ cm}^{-1}$ ) in fucoxanthin [(3*S*, 5*R*, 6*S*, 3'*S*, 5'*R*, 6'*R*)-5,6-epoxy-3,3',5'-tri-hydroxy-6',7'-didehydro-5,6,7,8,5'-hexahydro- $\beta$ , $\beta$ -caroten-8-one-3'-acetate] (1.57) and neoxanthin (1.47), a *cis*-double bond in *cis*-phytoene (1.34) (C-H



out of plane deformations of a *cis*-double bond at  $758\text{ cm}^{-1}$  compared with  $965\text{ cm}^{-1}$  for the *trans*-double bond), an asymmetrically substituted acetylene group in alloxanthin (1.46) (weak peak at  $2170\text{ cm}^{-1}$ ), and a carbonyl group ( $1600\text{--}1700\text{ cm}^{-1}$ ) in fucoxanthin; the infra-red spectrum of fucoxanthin (Fig. 1.4) illustrates a number of these points. Secondary and tertiary alcohols and methoxy groups found particularly in carotenoids from photosynthetic bacteria can also be quickly recognized [44].

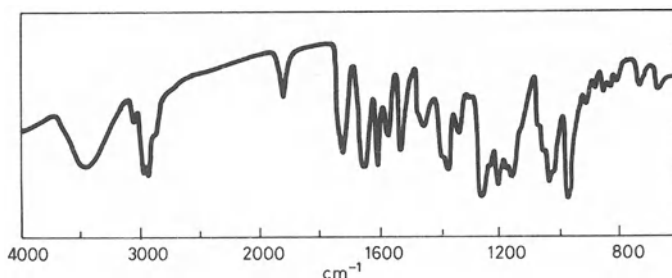
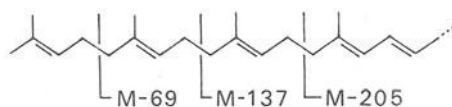


Fig. 1.4. Infrared spectrum of fucoxanthin (1.57). (Reproduced from [15]).

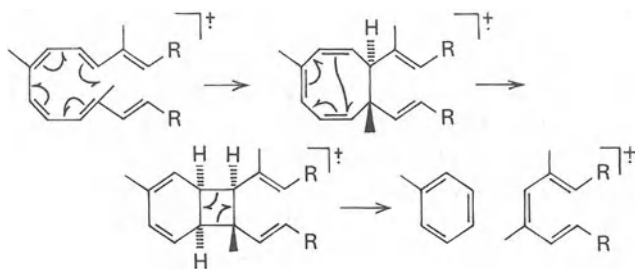
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### 1.4.3 MASS SPECTROMETRY (m.s.)

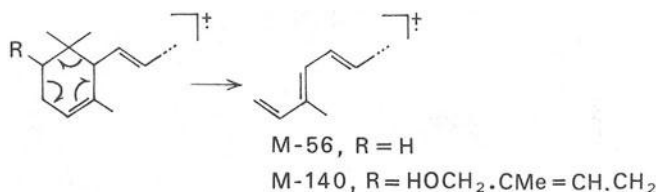
Mass spectrometry has had tremendous impact on the elucidation of carotenoid structures, particularly when frequently not more than 100  $\mu\text{g}$  of a pigment are required for analysis. Not only will a high resolution instrument give the molecular weight to within 2 p.p.m. but a study of the fragmentation pattern may lead quickly to a clear indication of the structure [15]. Again it would take too much space to discuss the mass spectra of carotenoids in detail here, but it is appropriate in passing to indicate some of the ways the technique has been used to establish difficult structures. Acetylenic carotenoids can be distinguished from their non-acetylenic counterparts, e.g. alloxanthin (1.46) and zeaxanthin (1.18) (difference in molecular ions). Bisallylic single bonds readily fragment with the loss of 69 137 and 205 mass units, so that in phytoene, for example, the position of the polyene chromophore can be clearly seen, thus:



A characteristic which can quickly indicate whether a pigment is or is not a carotenoid is the loss of  $\text{C}_7\text{H}_8$  (toluene, M-92),  $\text{C}_8\text{H}_{10}$  (xylene, M-106) and  $\text{C}_{12}\text{H}_{14}$  (M-158) thus:



Substitution at in-chain methyls can be located by the formation of derivatives of toluene etc. Typical fragmentation patterns are observed with epoxides, aromatic rings and  $\epsilon$ -rings. The fragmentation of the  $\epsilon$ -ring is as follows:



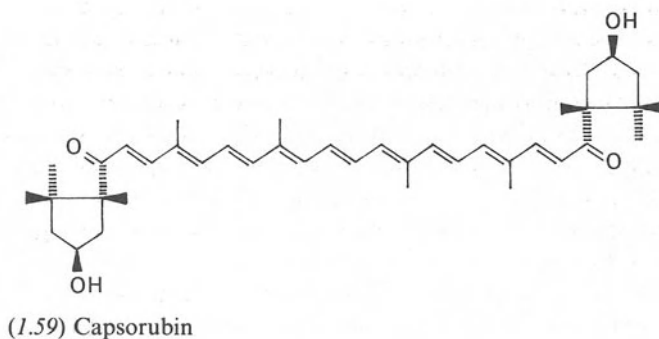
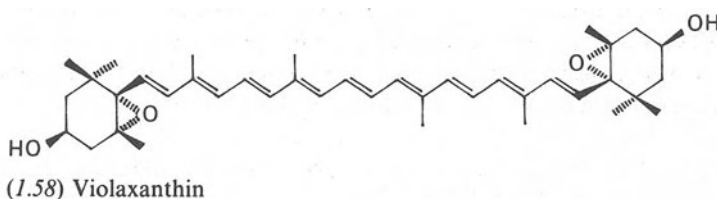
## 1.4.4 NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY (n.m.r.)

(a) *Proton magnetic resonance spectroscopy* (p.m.r.)

The spectra of many carotenoids run on a 60 or 100 MHz n.m.r. instrument with tetramethylsilane as reference have now been measured, and often the only easily recognizable signals for most of the pigments are the methyl singlets in the region of  $\delta$  0.7–3.8 p.p.m. Other signals which can be recognized are given by Moss & Weedon [15]. Many more useful data [15] have been obtained on a 220 MHz machine but it is not appropriate to discuss them here.

(b) *Carbon magnetic resonance spectroscopy* (C.m.r.)

New developments in pulsed Fourier transform techniques for n.m.r. spectroscopy allow the measurement of  $^{13}\text{C}$  spectra at natural abundance. The C.m.r. spectra of many simple carotenoids are now well described [45] and even in a carotenoid as complex as fucoxanthin, which contains 42 carbon atoms, 41 separate signals have been recorded and assigned [15].  $^{13}\text{C}$ .m.r. studies are also revealing the exact structures of some *cis*-isomers: for example, violaxanthin is the 9-*cis*-isomer of violaxanthin (1.58) (5,6,5',6'-diepoxyzeaxanthin) and the *neo*-A isomers of zeaxanthin (1.18) [46] and capsorubin (1.59) have the 13-*cis* configuration whilst *neo*-B isomers are 9-*cis* [47].



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### 1.4.5 OPTICAL ROTATORY DISPERSION (o.r.d.) AND CIRCULAR DICHROISM (c.d.)

Until recently, o.r.d. and c.d. measurements of carotenoids were confined to the ultra violet region because of the intense absorption of the compounds in the visible region of the spectrum. The technical problems associated with measurements in the visible region of the spectrum have now been solved and excellent spectra are available, as for example for violaxanthin (1.58) (Fig. 1.5). O.r.d. and c.d. spectra are useful for studies on absolute configuration. Furthermore, the spectra of carotenoid half molecules are additive within experimental error; for example,  $\beta$ -cryptoxanthin (1.60) (monochiral) has a very similar o.r.d. spectrum to that of zeaxanthin (1.18) (homo-

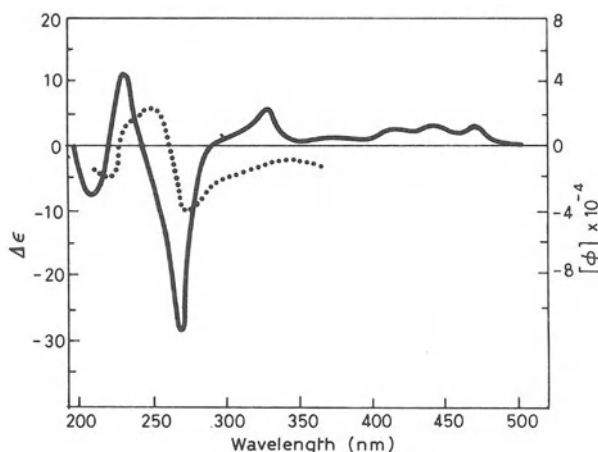
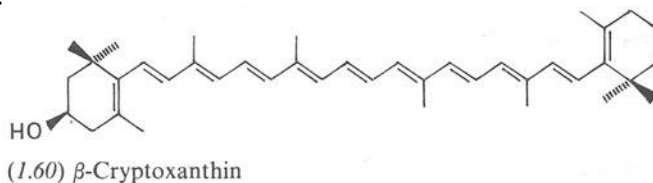


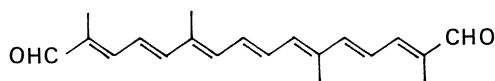
Fig. 1.5. O.r.d. in dioxan (.....) and c.d. in methanol (—) of violaxanthin (1.58). (Adapted from [15].)

dichiral) but with half the amplitude. Full details of o.r.d. and c.d. spectra of carotenoids have recently been reported [15].

### 1.4.6 X-RAY CRYSTALLOGRAPHY

Only three carotenoids have so far been examined by X-ray crystallography,  $\beta$ -carotene (1.13) [48], canthaxanthin (4,4'-diketo- $\beta$ -carotene) (1.40) [49] and crocetindial (1.61) [50]. The results confirm the conformation indicated on p. 28.





(1.61) Crocetindial

## 1.5 CHEMICAL PROPERTIES

The chemical properties of carotenoids have been discussed in detail elsewhere [10, 15–18, 75–77], so in this section only those properties which are diagnostically useful will be considered, together with a short discussion on the stereochemical aspects which are necessary for a full understanding of the biosynthetic problems discussed in Chapter 2.

### 1.5.1 REACTIONS WITH ACIDS

The 5,6-epoxides in the presence of HCl are rapidly isomerized to 5,8-epoxides (furanoid oxides); this change involves the shortening of the chromophore as in the conversion of violaxanthin (1.58) into auroxanthin (1.62) and is accompanied by a marked hypsochromic shift (Fig. 1.6). The shift is 7–22 nm for monoepoxides and about 40 nm for diepoxides, as in

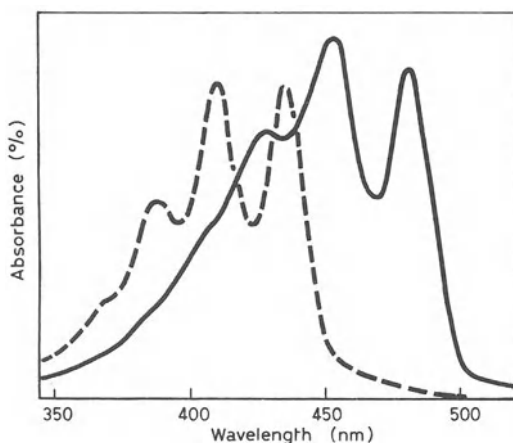


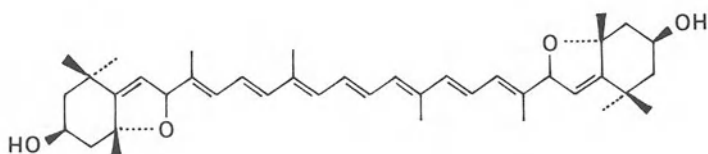
Fig. 1.6. Electronic spectral changes in benzene on conversion of violaxanthin (—) (1.58) into auroxanthin (----) (1.62) by hydrogen chloride [15].

the case illustrated. A test for the presence of an epoxide can conveniently be carried out directly in the cuvette of a spectrophotometer by adding a small drop of dilute HCl (0.1 M) to an ethanolic solution of the original pigment and noting the spectral change.

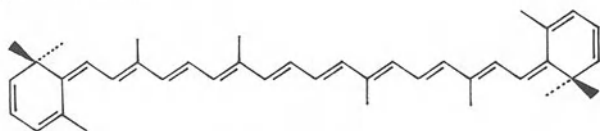
Treatment of allylic alcohols with 0.1 M hydrogen chloride in chloroform results in rapid dehydration, for example, eschscholtzxanthin (1.26) is con-

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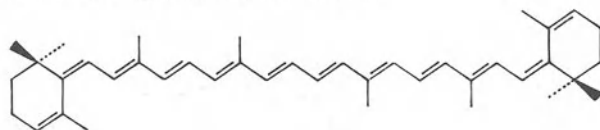
verted into its *bis*-anhydro derivative (1.63) [51]. In other cases, the proton is eliminated from the vinylogous position at the opposite end of the polyene chain so that, for example, isocryptoxanthin (1.35) is converted into the *retro*-derivative, *retro*-dehydro- $\beta$ -carotene (1.64) [52]. Finally, the elimination of an allylic function can result in another allylic system which further reacts with acid so that in the case of reduced spheroidenone (1.65) [53, 54] the product is 3,4-dehydrolycopene (1.15). All the cases just quoted are accompanied by changes in electronic spectra but the epimeric (C-8) fucoxanthols (1.66) undergo both allylic dehydration and epoxide isomerization to give the



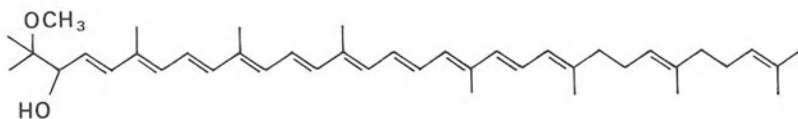
(1.62) Auroxanthin



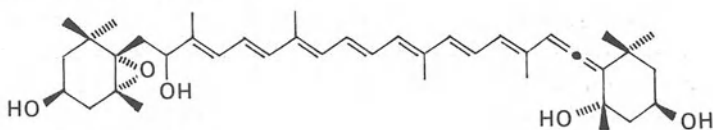
(1.63) Bisanhydroeschscholtzxanthin



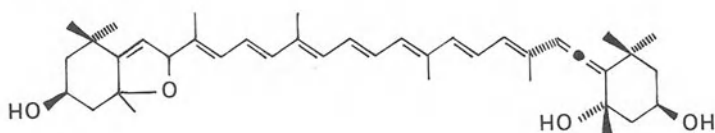
(1.64) Retrodehydro- $\beta$ -carotene



(1.65) Reduced spheroidenone



(1.66) Fucoxanthol

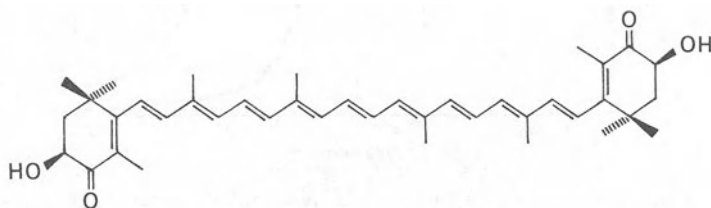


(1.67) Fucochrome

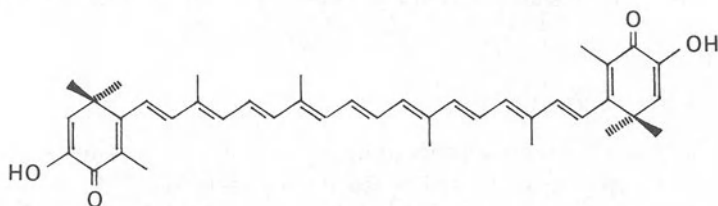
fucochromes (1.67) with the result that there is no net change in absorption spectrum. The change in structure can, however, be readily detected by noting the change in adsorption properties on thin-layer chromatography [55, 56].

### 1.5.2 REACTIONS WITH BASES

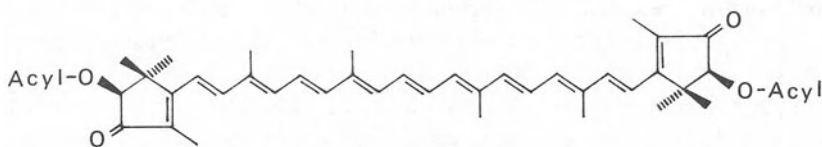
Most carotenoids are stable to base hence the common use of saponification as an early step in the purification process. However, there are exceptions: (i) astaxanthin (1.68) is rapidly oxidized in air to astacene (1.69); (ii) actinioerythrin (1.70) is autooxidized to a variety of products [57]; (iii) peridinin (1.71) and fucoxanthin (1.57) are both unstable to base even under anaerobic conditions [58]. On the other hand, polyene aldehydes are unexpectedly stable to base even in the presence of oxygen but in the presence of acetone and base will give rise by an aldol condensation to  $\beta$ -hydroxy ketones which readily dehydrate to enones. This change is characterized by a marked shift in absorption spectrum resulting from an extension of the carbon-carbon polyene system. This is illustrated in Fig. 1.7 when crocetindial (1.61) (9 conjugated double bonds) is converted into the ketone (1.72) with 11 such bonds. The reaction is so rapid even at room temperature that acetone as an extracting solvent is not recommended; artefacts have been reported as naturally occurring products particularly in citrus fruit (see Chapter 5).



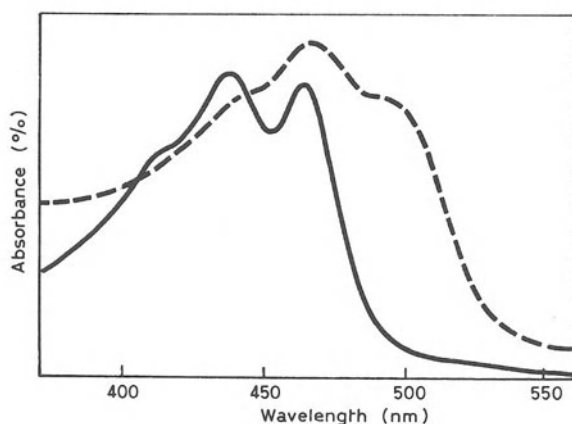
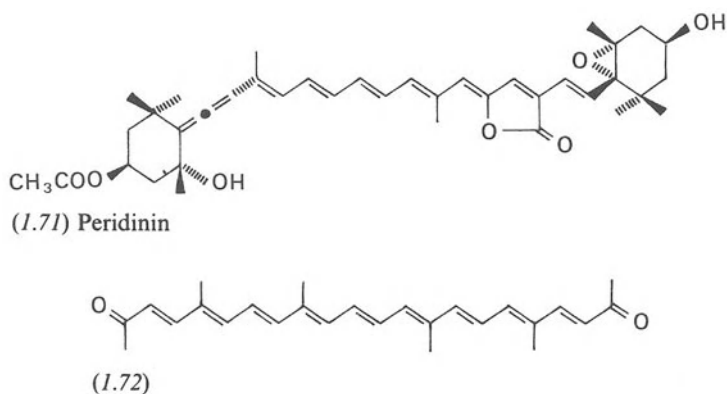
(1.68) Astaxanthin



(1.69) Astacene



(1.70) Actinioerythrin



*Fig. 1.7.* Electronic spectral changes on conversion of crocetindial ( $C_{20}$ ) (1.61) (—) in acetone into the  $C_{26}$  diketone (1.73) (----) following addition of 3% ethanolic potassium hydroxide [15].

### 1.5.3 HYDRIDE REDUCTIONS

Treatment of a carotenoid containing a conjugated carbonyl group with sodium borohydride in ethanol or tetrahydrofuran will result in a dramatic change in absorption spectrum. There is a hypsochromic shift of 20–30 nm and an enhancement of the fine structure of the spectrum. This is well illustrated in the case of spheroidenone (1.42) (Fig. 1.8). Reduced spheroidenone (spheroidenol) has the same spectrum as that of spheroidene (1.41) which is given in Fig. 1.8 [58].

As is the case with the HCl test, outlined in Section 1.5.1, the reaction can be carried out in a cuvette and is extremely useful diagnostically. If lithium aluminium hydride is used instead of sodium or potassium boro-



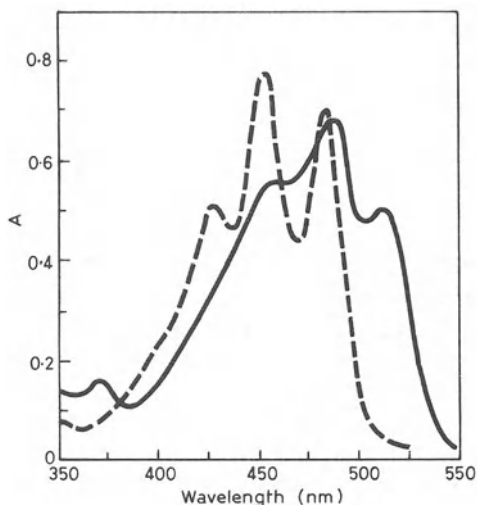


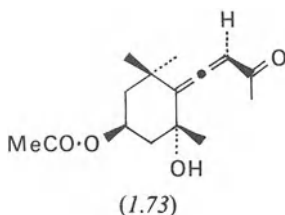
Fig. 1.8. Electronic absorption spectra in light petroleum of spheroidene (-----) (1.41) and spheroidenone (——) (1.42) [29].

hydride a complex is formed which needs to be destroyed by the addition of water. Furthermore, with the latter reagent only aldehydes and ketones are reduced, whereas with lithium aluminium hydride carotenoic acids and their esters are reduced in addition to the aldehydes and ketones.

#### 1.5.4 STEREOCHEMISTRY—ABSOLUTE CONFIGURATION

Some information on the absolute configuration of carotenoids is necessary in order to understand the biosynthetic subtleties of their formation (Chapter 2). The first pigment for which the absolute configuration at any chiral centre was elucidated was capsanthin (5*R*) (1.30) following degradation to derivatives of natural camphor [59]. Later, the full stereochemistry (3*R*,3'*S*,5'*R*) was demonstrated [60] which showed that the absolute configuration at C-3 was the same in the  $\beta$ - and  $\kappa$ -rings.\* Synthesis of capsorubin (1.59) from (+) camphor revealed that the same chirality existed at both ends of the molecule [61]. X-ray crystallographic studies on (1.73), a key degradation product of fucoxanthin, demonstrated that the allenic end groups in fucoxanthin (1.57) and neoxanthin (1.47) must have the 3'*S*,5'*R*,6'*R* configuration as indicated in (1.73) [62]. As a correlation had already been established between fucoxanthin (1.57), zeaxanthin (1.18), violaxanthin (1.58)

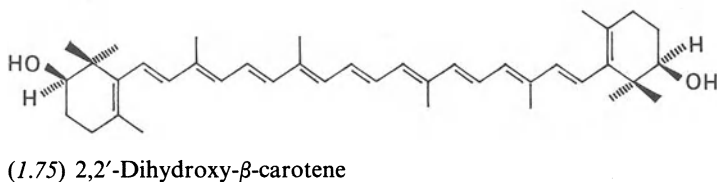
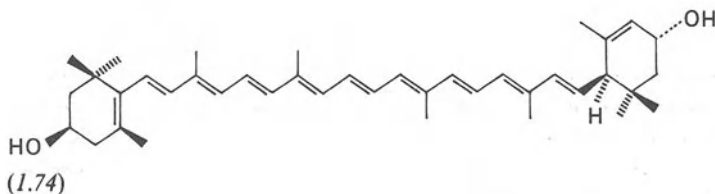
\* Note that the priority rules of the *R,S* convention demand that the chiral centre as C-3' be designated *S* although it has the same absolute configuration as C-3 which is designated *R*.

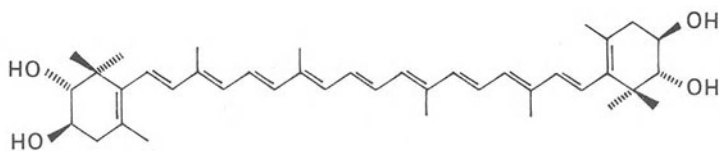
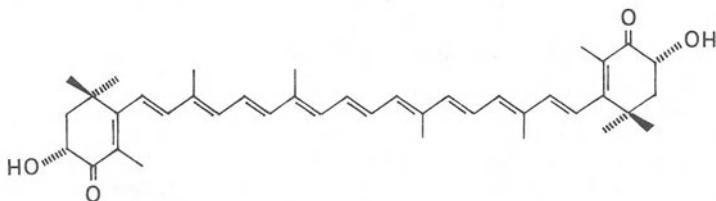
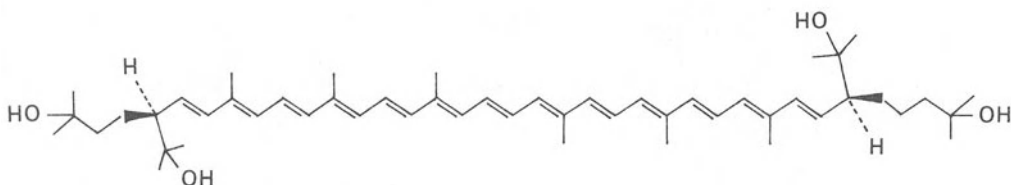


and alloxanthin (1.46) it follows that all these pigments have the same configuration at C-3 and C-3'. The configuration of the 5,6-epoxy end group of violaxanthin (1.58), lutein epoxide (1.48) and neoxanthin (1.47) was shown to be 5*R*,6*S* by converting the compounds into their 5,8-furanoid oxides and examining the n.m.r. properties of the epimers resulting from the production of an additional chiral centre at C-8 [56]; the 5,6-epoxy group has the same chirality in fucoxanthin (1.57) [62].

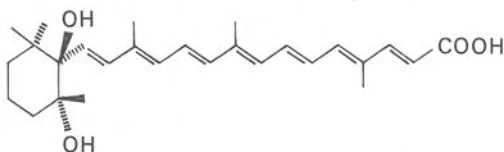
The configuration at C-6' in *α*-carotene (1.12) and other compounds, e.g. lutein, which contain the  $\epsilon$ -end group has been shown to be *R* by comparison with manool and ambrein [63–65]. A further important finding was that the chirality at C-3' in lutein (1.74) is *R* which is opposite to that at C-3 in zeaxanthin and, indeed, at C-3 in lutein itself [64, 66].

The recently discovered carotenoids with hydroxyls at C-2 in certain Chlorophyta (see Chapter 7), e.g. (1.75), have the 2*R*,2'*R* configuration [67]. However, it cannot be assumed that the chirality of similar carotenoids from different organisms is always the same;  $\beta,\beta$ -carotene-2,3,3'-triol and  $\beta,\beta$ -carotene-2,2',3,3'-tetrol (1.76) from the Cyanophyta both have a configuration at C-2 and C-2' opposite to that of the corresponding 2-diol and 2,2'-diol from the Chlorophyta [68]. However, in all cases the assignment is 2*R* (see footnote on p. 27). Another example is that astaxanthin from the yeast *Phaffia rhodozyma* has the 3*R*,3'*R* configuration [(1.77), compare (1.68)] [69] opposite to that in astaxanthin and zeaxanthin from all other sources so far examined.



(1.76) 2,3,2',3'-Tetrahydroxy- $\beta$ -carotene(1.77) Astaxanthin ex *Phaffia rhodozyma*

(1.78) Bacterioruberin



(1.79) Azafrin

The absolute configurations of the  $C_{50}$  carotenoids bacterioruberin (2*S*,2'*S*) (1.78) [70] and decaprenoxanthin (2*R*,6*R*,2'*R*,6'*R*) (1.29) [71] and the apocarotenoid azafrin (5*R*,6*R*) (1.79) [72] are also known.

## 1.6 ADDENDUM

Annual reviews of advances in carotenoid chemistry are now appearing in a publication of the Chemical Society [16–18, 75–77].

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## BIOSYNTHESIS OF CAROTENOIDS

## 2.1 INTRODUCTION

Carotenoids, being terpenoids, are synthesized from the basic  $C_5$  terpenoid precursor, isopentenyl pyrophosphate (IPP). The first part of the chapter is concerned with evidence for IPP formation in higher plants and carotenogenic micro-organisms and for its conversion into geranylgeranyl pyrophosphate (GGPP), the  $C_{20}$  precursor of phytoene, the first  $C_{40}$  hydrocarbon formed. Then the formation of phytoene is discussed followed by a full description of its conversion into lycopene. Finally, the conversion of lycopene and related compounds, such as neurosporene, into the various naturally occurring carotenes and xanthophylls is described. This should allow the reader to follow more easily specialized aspects of biosynthesis, such as intracellular location of synthesis and photoinduction of synthesis, in the appropriate chapters which follow.

## 2.2 GENERAL PATHWAY TO LYCOPENE

## 2.2.1 FORMATION OF ISOPENTENYL PYROPHOSPHATE

The universal  $C_5$  biological isoprene precursor is isopentenyl pyrophosphate (IPP) (2.1) but the first specific precursor on the terpenoid biosynthetic pathway is the  $C_6$  compound mevalonic acid (MVA) (2.2). Even before MVA was discovered experiments with  $[1-^{14}C]$ acetate and  $[2-^{14}C]$ acetate had shown that a mechanism involving three acetate units (Fig. 2.1) was probably functioning in isoprenoid biosynthesis, and in the carotenoid field the utilization of  $[^{14}C]$ acetate for  $\beta$ -carotene synthesis was first demonstrated in the fungi *Phycomyces blakesleeanus* [1-5] and *Mucor hiemalis* [5, 6]. Degradation of the  $[^{14}C]$  $\beta$ -carotene so formed revealed a labelling pattern (Fig. 2.2) consistent with the repeating pattern of a  $C_5$  unit envisaged in Fig. 2.1. Similar results were obtained in other laboratories with *P. blake-*

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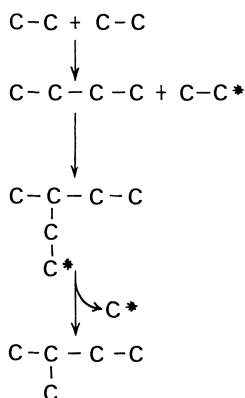
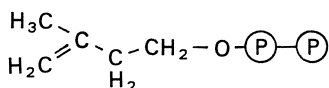
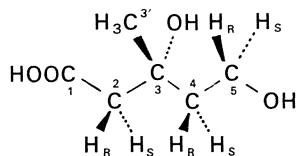


Fig. 2.1. Production of a C<sub>5</sub> branched unit from three C<sub>2</sub> units.



(2.1) Isopentenyl pyrophosphate



(2.2) Mevalonic acid

*sleeaanus* [7–9], *Euglena gracilis* [10] and carrot root slices [9, 11]. Following the discovery that the naturally occurring 3*R* enantiomer of MVA (2.2) [12] was a precursor of sterols in animals [13] it was not long before [2-<sup>14</sup>C]MVA

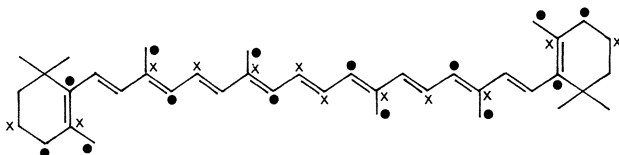


Fig. 2.2. Labelling pattern in  $\beta$ -carotene formed from [<sup>14</sup>C]acetate.

((●) carbons from C-2 of acetate; (x) carbons from C-1 of acetate)

was shown to be a precursor of carotenoids in *P. blakesleeaanus* [7–9, 14, 15], *M. hiemalis* [16], *Blakeslea trispora* [17], *Neurospora crassa* [18], carrot root preparations [9, 19], tomato fruit [7–9, 19–21], *Euglena gracilis* [10] and in a mutant of the bacterium *Staphylococcus aureus* [22]. However, it is not always easy to demonstrate incorporation of MVA in intact organisms because of permeability problems; such is the case with *Chlorella pyrenoidosa* and various strains of *E. gracilis* [10, 23] and to some extent with greening etiolated seedlings [24–26] (see also p. 121). The pathway from acetyl-CoA to MVA via 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) was quickly established in animals and frequently confirmed, but it has been much less well documented in plants. The enzymes involved in HMG-CoA synthesis,



acetoacetyl-CoA-thiolase and HMG-CoA synthase (Fig. 2.3), have yet to be demonstrated in higher plants although the latter has been purified from yeast [27, 28]. Only recently has the key enzyme HMG-CoA reductase been

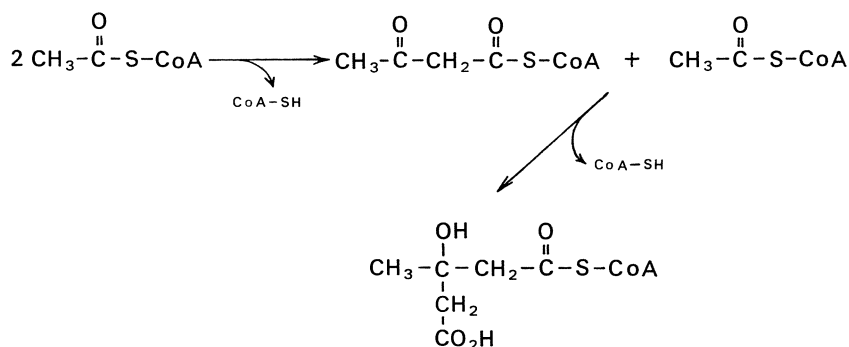


Fig. 2.3. Formation of  $\beta$ -hydroxy- $\beta$ -methylglutaryl-CoA (HMG-CoA) from acetyl-CoA.

detected in higher plants [29, 30] and algae [31], although it had earlier been shown to be present in the non-carotenogenic rubber latex [32]. It has been claimed that HMG itself is an active carotenoid precursor in preparations from *P. blakesleeanus* [14, 15]. This implies an HMG-activating enzyme which

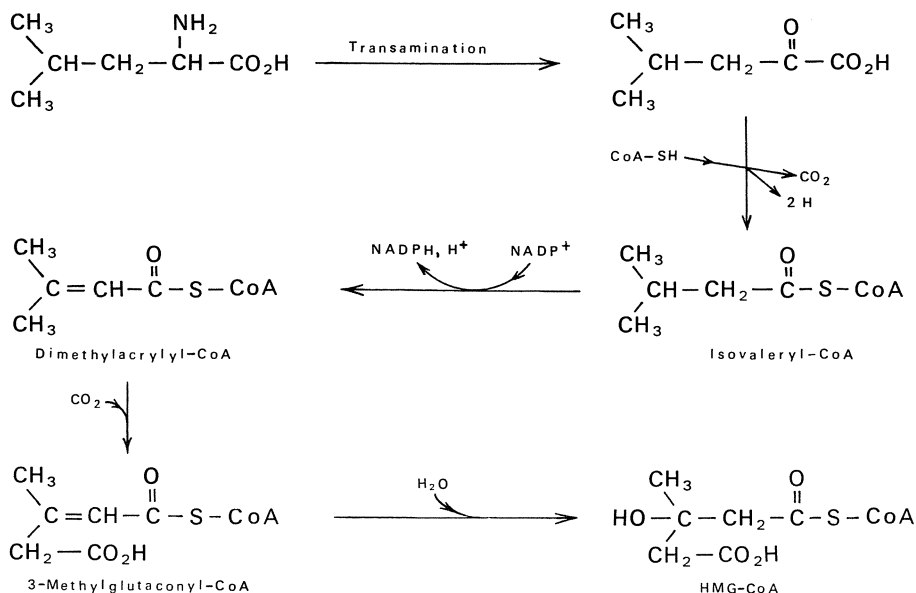


Fig. 2.4. The conversion of leucine into HMG-CoA.

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is not widely distributed; indeed in *E. gracilis* HMG is transported into the cell but not metabolized [10].

An alternative pathway to HMG-CoA and thus to MVA exists in *P. blakesleeanus* in which the amino acids valine and leucine are extremely carotenogenic [33]. The pathway from leucine to HMG-CoA was worked out in yeast (Fig. 2.4) [34] and radioisotope experiments indicate that the same pathway is functioning in *P. blakesleeanus* [15, 35–40]. The mechanism outlined involves fixation of CO<sub>2</sub> and it is interesting that <sup>14</sup>CO<sub>2</sub> is incorporated into β-carotene by *P. blakesleeanus* only when it is metabolizing leucine. However, the mechanism involves CO<sub>2</sub> fixation into C-1 of MVA (Fig. 2.4) which is lost when MVA is converted into IPP. The scheme outlined in Fig. 2.5 indicates known pathways which will allow the conservation of some of the fixed CO<sub>2</sub> in C-5 of MVA. [<sup>14</sup>C]Leucine is also incorporated

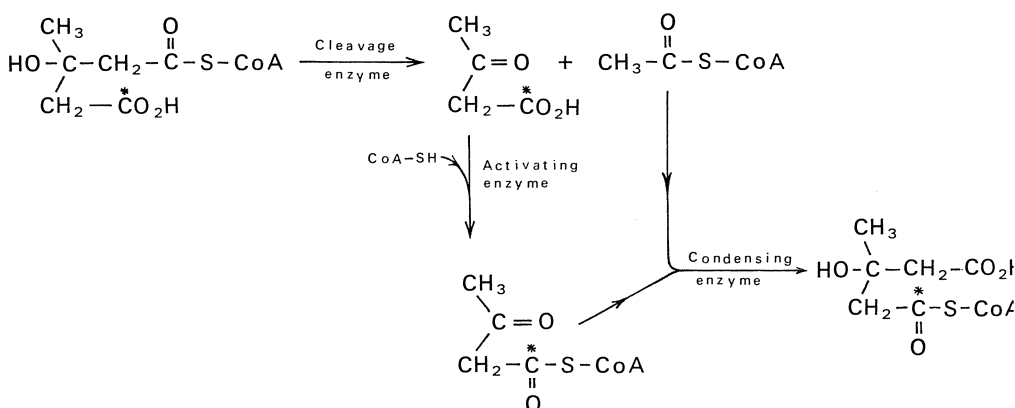


Fig. 2.5. The breakdown and resynthesis of HMG-CoA necessary to account for the incorporation of <sup>14</sup>CO<sub>2</sub> into β-carotene in the presence of unlabelled leucine.

into carotenoids in green tissue of higher plants [41]. However, the unlabelled acid is not stimulatory in *Rhodospirillum rubrum* [42], *Corynebacterium poinsettiae* [43] or *Sarcina lutea* [44]. In *Rhodotorula shibatana* leucine increased carotenoid synthesis but oddly enough valine, which can also give rise to HMG-CoA, did not [45, 46]. Of the intermediates involved in leucine metabolism only dimethylacrylic acid, and not its activated form (CoA-ester), has been examined as a possible carotenoid precursor. Results were equivocal; it has been reported to be both stimulatory [47] and non-stimulatory [33] in *P. blakesleeanus*. [3-<sup>14</sup>C]Dimethylacrylic acid was incorporated into carotenoids in *Euglena gracilis* [10], *Chlorella pyrenoidosa* [17] and *Blakeslea trispora* [17], but not in tomato fruit under conditions which allowed MVA to be incorporated [17].

MVA is converted into IPP by a series of reactions indicated in Fig. 2.6. The first reaction involving MVA kinase has been demonstrated in a number

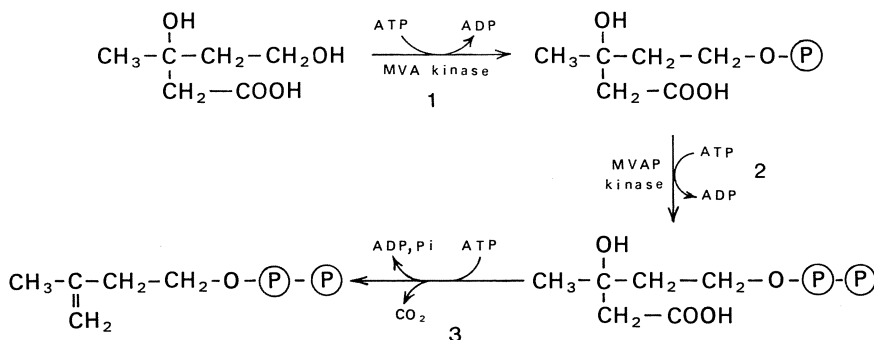


Fig. 2.6. Steps in the formation of IPP from MVA.

of plants and micro-organisms including seedlings of *Cucurbita pepo* [48], *Pinus radiata* [49] and *P. pinaster* [50], cotyledons of *Cucumis melo* [51], tissue cultures of *Kalanchoe crenata* [52], *Hevea brasiliensis* latex [53, 54], seeds of *Pisum* [55], leaf tissue and etiolated cotyledons of *Phaseolus vulgaris* [56, 57], orange juice vesicles [58] and *Staphylococcus aureus* [59]. MVA-5-phosphate kinase has been demonstrated in yeast [60–62] and the product, MVA-5-pyrophosphate, has been identified as an intermediate in a bacterial system converting MVA into carotenes [63] and a cell-free system from *Agave americana* carries out both steps 1 and 2 (Fig. 2.6) [64] as do preparations from *C. pepo* [48], *P. radiata* [49], *P. pinaster* [50], *Pisum* [55], orange juice vesicles [58], callus cultures of *K. crenata* [52] and *S. aureus* [59]. MVA-5-pyrophosphate anhydrodecarboxylase (3, Fig. 2.6) has been purified from yeast [60–62, 65] and the latex of *H. brasiliensis* [66]. IPP is present in various systems which can synthesize carotenoids [67] and is converted into carotenoids in many preparations including those from tomato fruit [68], *S. aureus* [67], fruit plastids [68–70], chloroplasts [68–70], *Phycomyces blakesleeana* [71, 72] and *Neurospora crassa* [72A].

### 2.2.2 FORMATION OF PHYTOENE

In the process of chain elongation, i.e.  $C_5 \rightarrow C_{10} \rightarrow C_{15}$  etc., the first step is the isomerization of IPP to dimethylallyl pyrophosphate (DMAPP) (Fig. 2.7), which then acts as starter for chain elongation [73, 74]. The enzyme prenyl transferase condenses one IPP molecule with a DMAPP molecule to form the  $C_{10}$  geranyl pyrophosphate [68, 75–77]. The sequential transfer of two further IPP molecules yields first farnesyl pyrophosphate (FPP,  $C_{15}$ ) and then geranylgeranyl pyrophosphate (GGPP,  $C_{20}$ ) [78–82]. It appears that one enzyme with two active sites is involved in the synthesis of FPP [83] although two separate forms are present in germinating rice seedlings [84]. GGPP has been obtained from  $C_5$ ,  $C_{10}$  and  $C_{15}$  precursors in preparations from yeast [85], carrot root [86], tomato root plastids [68],

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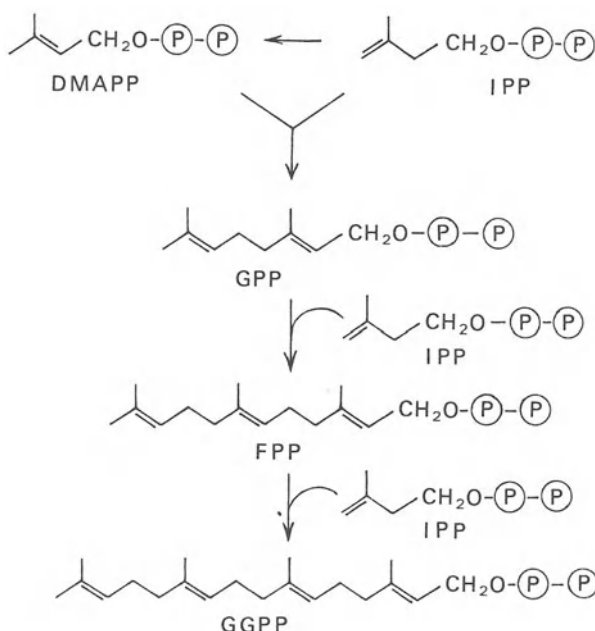


Fig. 2.7. The conversion of IPP into GGPP.

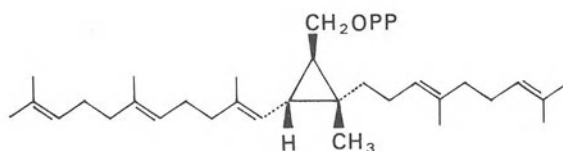
pumpkin fruit [87], liquid endosperm of *Echinocystis macrocarpa* [88], proplastids from germinating castor bean endosperm [170] and *Micrococcus lysodeikticus* [79].

All the pyrophosphate intermediates just discussed have been implicated, either directly or indirectly, in carotenoid biosynthesis. The presence of IPP isomerase has been demonstrated in a carotenogenic preparation from tomato plastids [68] and two isoenzymes have been obtained from pumpkin fruit [89]. The direct incorporation of DMAPP into carotenoids has, however, not yet been reported although the inhibition of the conversion of MVA into carotenoids by iodoacetamide in a crude cell-free system from *P. blakesleeana* is circumstantial evidence for DMAPP being an intermediate; the IPP-DMAPP isomerase is an -SH enzyme [89].

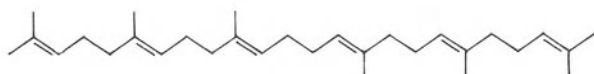
Utilization of GPP for carotenogenesis has also yet to be directly demonstrated but GPP is formed by preparations from seedlings of pea [54], *Micrococcus lysodeikticus* [89A] and *Pinus radiata* [50]. Free geraniol has been incorporated into  $\beta$ -carotene by carrot root slices [90] presumably because of the presence of enzymes which will phosphorylate geraniol; in this aspect of terpenoid biosynthesis higher plants are unlike animals and fungi which do not appear to produce such phosphorylating enzymes. FPP is incorporated into  $\beta$ -carotene and other polyenes in a cell-free system from *P. blakesleeana* [91] and in plastid systems from carrots and tomatoes [92, 93]. Early work implicated GGPP in carotenogenesis because, in the presence of FPP, MVA,

as a source of the additional IPP residue necessary for the conversion of FPP into GGPP, was a mandatory requirement for phytoene synthesis in a cell-free system from *P. blakesleeana* [91]. Similarly, in isolated tomato plastids IPP was necessary in addition to FPP for carotenoid synthesis [69]. Later experiments have directly demonstrated conversion of GGPP into phytoene by enzyme systems from tomato plastids [70], *P. blakesleeana* [71] and chloroplasts [94].

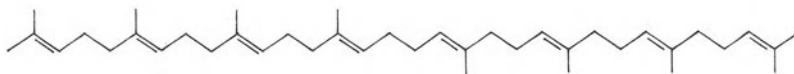
In the presence of squalene synthase and NADPH two molecules of farnesyl pyrophosphate condense to form presqualene pyrophosphate (2.3) which is then converted into squalene (2.4), the  $C_{30}$  acyclic precursor of sterols [95, 96]. An analogous reaction with GGPP would produce the  $C_{40}$  compound lycopersene (2.5) which does not appear to be a natural product although it is said to be formed non-specifically from GGPP in the presence of squalene synthase. A much more likely carotenoid precursor is phytoene (1.36) (15,15'-dehydrolycopersene) and there is now substantial evidence for this view. (i) It is formed from MVA by isolated chloroplasts [97] and by a cell-free system from peas [98], and from GGPP by a soluble system of tomato fruit plastids [70, 99] without any indication of lycopersene formation. (ii) NADPH is not required as a co-factor for phytoene synthesis as it is



(2.3) Presqualene pyrophosphate



(2.4) Squalene

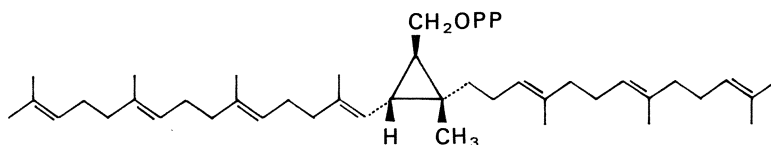


(2.5) Lycopersene

in the formation of squalene and would be if lycopersene were the primary product, and addition of NADPH to the chloroplast and tomato plastid systems merely resulted in slight stimulation of squalene synthesis. (iii) If a *Mycobacterium* sp. is cultured anaerobically in the absence of a hydrogen acceptor phytoene and not lycopersene accumulates [100]. (iv) In preparations from a *Mycobacterium* sp. and a phytoene-accumulating mutant of *P. blakesleeana* the phytoene formed in the presence of  $[4-^{14}C, 1-^3H_2]$  GGPP

retained two of the four labelled hydrogen atoms available. If lycopersene had been an intermediate, there should have been some dilution of the label at C-15 and C-15' by hydrogen from NADPH; furthermore, in the same system the presence of  $[4-^3\text{H}_2]\text{NADPH}$  did not result in the production of tritiated phytoene [101]. (v) Many mutants of *Chlorella vulgaris* [102], *C. pyrenoidosa* [103], *Neurospora crassa* [104], *Rhodospseudomonas sphaeroides* [105] and *Phycomyces blakesleeanus* [106] have been described which accumulate phytoene, but no mutants are known which accumulate lycopersene. (vi) When the desaturation process leading to coloured carotenoids is inhibited by diphenylamine (DPA) [107, 108] or San 6706 [109–111] then, again, phytoene and not lycopersene accumulates.

The intermediate between GGPP and phytoene is prephytoene pyrophosphate (PPPP)\* (2.6) [112, 113] which is the  $\text{C}_{40}$  analogue of presqualene



(2.6) Prephytoene pyrophosphate

pyrophosphate (2.3). The mechanism for the formation of this compound (Fig. 2.8) is the same as that involved in the formation of presqualene pyrophosphate [114]. The mechanism proposed would give rise to prephytoene pyrophosphate with the same chirality as that of presqualene pyrophosphate (1*R*,2*R*,3*R*) and optical properties of PPPP do indicate the same chirality [113]. However, in the conversion of PPPP into phytoene, the final step differs from that involved in the formation of squalene in which the intermediate cyclopropylcarbiny cation is stabilized by the addition of a hydride ion from NADPH. If this were the case in the carotenoid pathway the result would be lycopersene (a) and not phytoene (b) (Fig. 2.8). Phytoene would be formed by loss of a proton from the carbon atom which becomes C-15 of the phytoene. Which proton is lost depends on whether 15-*cis*- or 15-*trans*-phytoene is the final product formed (see Section 2.2.3).

The conversion of IPP into phytoene is carried out by an enzyme complex, molecular weight 200 000, which requires  $\text{Mn}^{2+}$  as co-factor and which is stimulated by ATP which possibly acts as an allosteric effector [115].

\* This has also been called prelycopersene pyrophosphate; although technically correct this name is biosynthetically confusing.

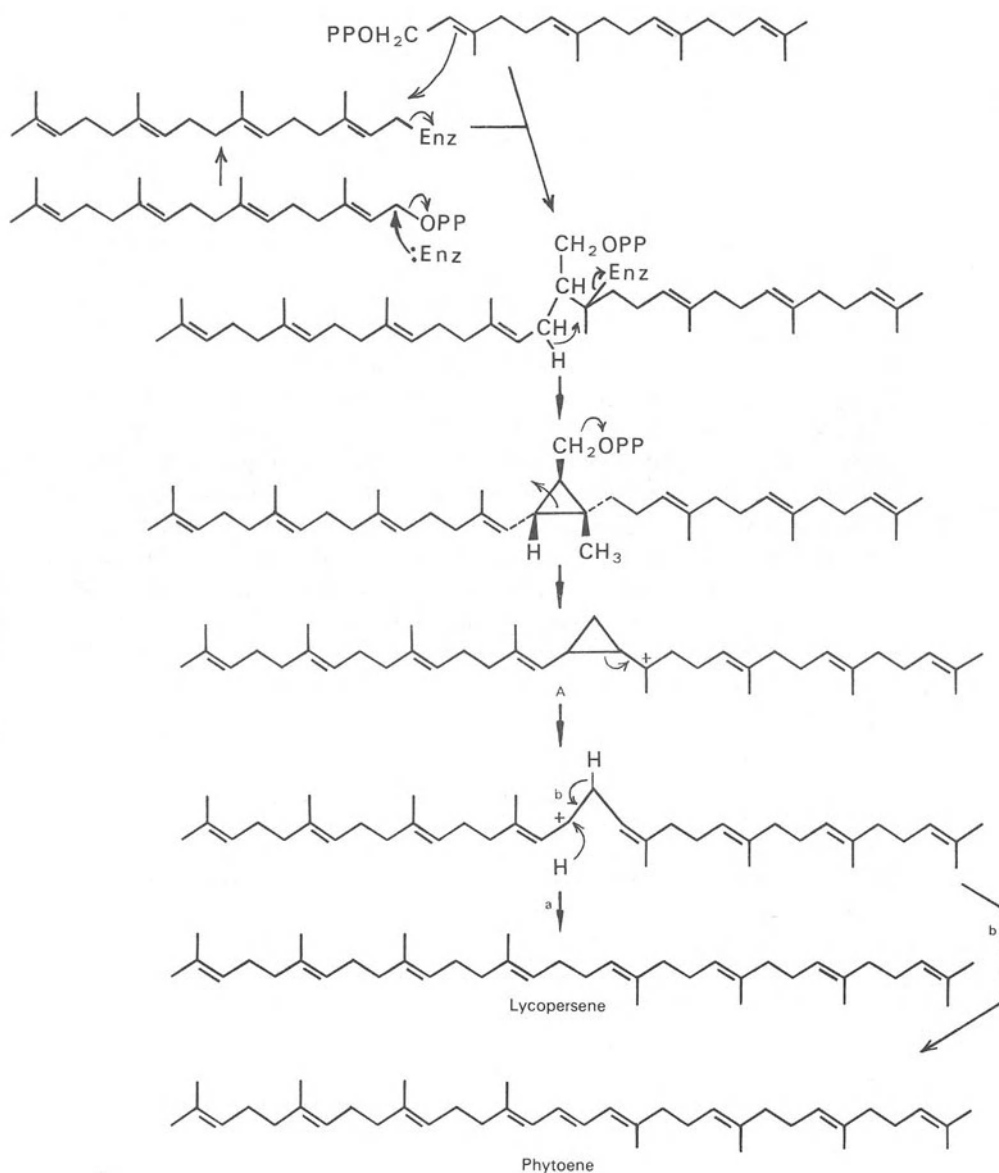
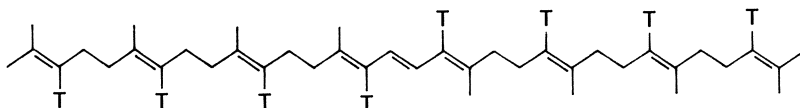


Fig. 2.8. A mechanism for conversion of prephytoene pyrophosphate into phytoene.

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### 2.2.3 STEREOCHEMISTRY OF PHYTOENE FORMATION

Mevalonic acid (MVA) (2.2) has three prochiral centres at C-2, C-4 and C-5.\* In the biosynthetic sequence leading to carotenoids there are several reactions in which a carotenogenic system has the choice of removing either the *pro-R* or *pro-S* hydrogen from each of these three centres. Chemically the two hydrogens at each centre are indistinguishable but enzymically they are likely to retain their spatial individuality when attached to the asymmetric enzyme surface. The problem was to test this idea experimentally and it was solved when Cornforth and Popják [95, 117, 118] and their colleagues brilliantly synthesized the six species of MVA stereospecifically labelled with either deuterium or tritium and tested them in appropriate biochemical systems. The first experiments were carried out with [(4*R*)-4-<sup>2</sup>H<sub>1</sub>]- and [(4*S*)-4-<sup>2</sup>H<sub>1</sub>]-MVA (2.2) which were incubated separately with a liver enzyme to yield FPP (C<sub>15</sub>). In the case of [(4*S*)-4-<sup>2</sup>H<sub>1</sub>]MVA the farnesol obtained contained no deuterium whilst in the case of [(4*R*)-4-<sup>2</sup>H<sub>1</sub>]MVA a trideuterated species of farnesol was obtained. This meant that the same hydrogen (*pro-S*) was eliminated in each of the three steps leading to FPP [118]. The same results were obtained when [2-<sup>14</sup>C,(4*R*)-4-<sup>3</sup>H<sub>1</sub>]- and [2-<sup>14</sup>C,(4*S*)-4-<sup>3</sup>H<sub>1</sub>]MVA were used, that is with the former the <sup>14</sup>C:<sup>3</sup>H ratio was the same in FPP as in the MVA used whilst with the latter no tritium was present in the FPP [95]. The same results were obtained with squalene (the C<sub>30</sub> precursor of sterols) formed from two FPP molecules [95] and with phytoene formed from two molecules of GGPP [119]. The labelling pattern in phytoene when [2-<sup>14</sup>C,(4*R*)-4-<sup>3</sup>H<sub>1</sub>]MVA is the substrate is thus:



So the mechanisms of the reactions outlined in Figs. 2.6 and 2.7 can now be indicated with full stereochemistry in Fig. 2.9 (MVAPP anhydrodecarboxylase), Fig. 2.10 (IPP-DMAPP isomerase) and Fig. 2.11 (prenyl transferase). From Fig. 2.10 it will be seen that the face of the double bond (*re, re*) (see [116]) which is attacked by the incoming proton is now known [120].

In considering the final step in the production of phytoene note has to be taken of the natural occurrence of both all-*trans*-phytoene and 15-*cis*-

\* From the present point of view a prochiral centre has two identical substituents (hydrogens in the case of C-2, C-4 and C-5 of MVA). If one of the two atom substituents (hydrogens) at such a centre, which are chemically indistinguishable, is replaced by deuterium, for example, then a chiral centre is established. The hydrogen which when replaced by another substituent results in *S* chirality is known as the *pro-S* hydrogen. In the same way when the *pro-R* hydrogen is replaced by another substituent *R* chirality results. The hydrogens are designated H<sub>S</sub> and H<sub>R</sub> as in (2.2). For further discussion of prochirality in biochemistry see reference [116].



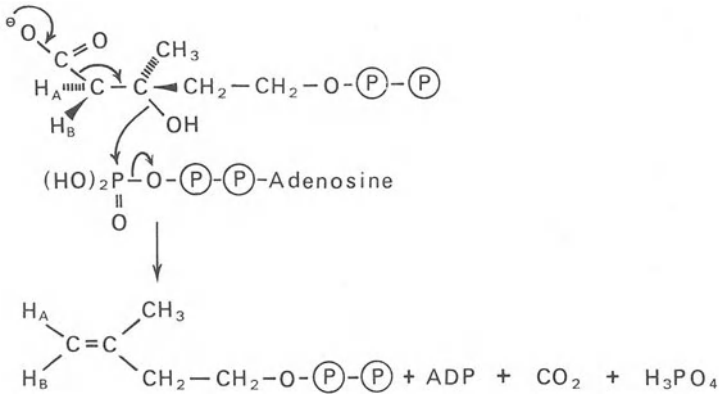


Fig. 2.9. Stereochemistry of formation of IPP from MVAPP.

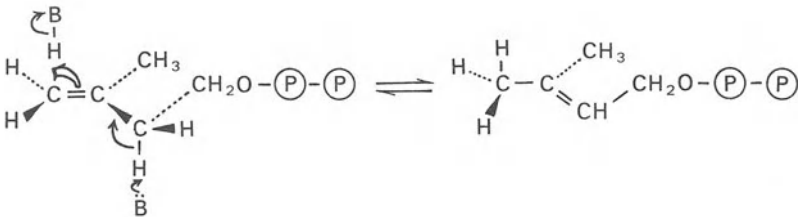


Fig. 2.10. The stereochemistry and mechanism of the  $\text{IPP} \rightleftharpoons \text{DMAPP}$  reaction.

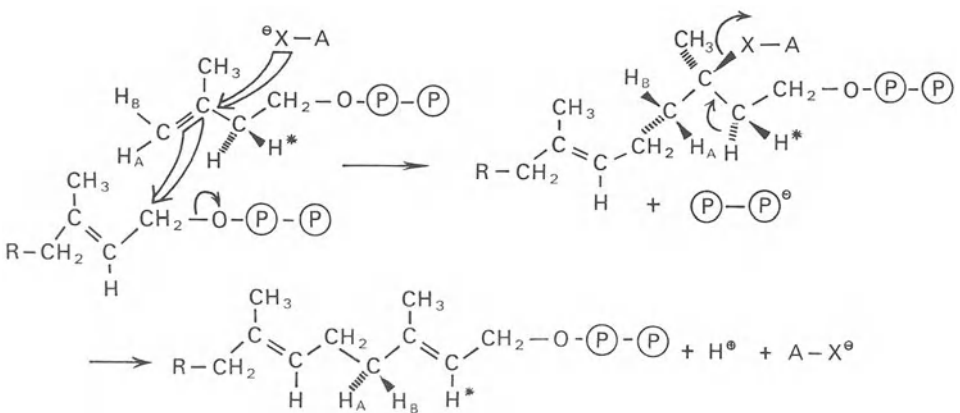


Fig. 2.11. Stereochemistry of hydrogen removal and mechanism of prenyl transferase reaction.

phytoene. The major component in higher plants, algae, fungi and photosynthetic bacteria is generally the *cis*-isomer whilst in many non-photosynthetic bacteria the *trans*-isomer predominates (Table 2.1). The question is whether both are formed biosynthetically as primary products or whether only one is formed directly from PPPP and is then isomerized into the other. If one considers further the mechanism outlined in Fig. 2.8 for phytoene formation from prephytoene pyrophosphate then, starting from the intermediate A, loss of the *pro-R* hydrogen would yield all-*trans*-phytoene and of the *pro-S* hydrogen *cis*-phytoene (Fig. 2.12). The carbon atom involved arises originally

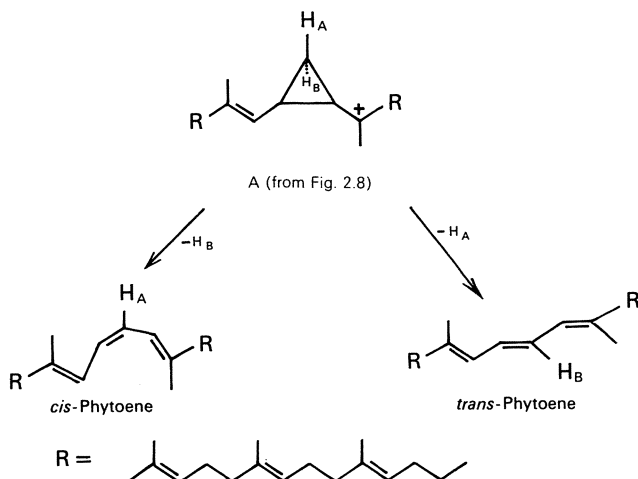


Fig. 2.12. Mechanism for formation of *cis*- and *trans*-phytoene by stereospecific loss of hydrogen from a common intermediate formed from prephytoene pyrophosphate (compare Fig. 2.8).

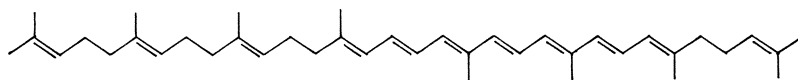
from C-5 of MVA and by using  $[2-^{14}\text{C}, (5R)-5-^3\text{H}_1]\text{MVA}$  it was shown that in tomato slices [132] and chloroplasts [133] formation of *cis*-phytoene resulted in loss of the 5-*pro-S* hydrogens from C-15 and C-15'. This was later confirmed by incubating  $[4-^{14}\text{C}, (1S)-1-^3\text{H}_1]\text{GGPP}$  (C-1 of GGPP arises from C-5 of MVA) with a preparation from *Phycomyces blakesleeanus*; no tritium was retained in the resulting *cis*-phytoene [101]. In contrast, the same experiment with a preparation from a *Mycobacterium* sp. yielded *trans*-phytoene with one 1-*pro-S* hydrogen (tritium) retained [101]. These experimental results lead to the mechanism proposed in Fig. 2.12 and also allow one to conclude that the hydrogen eliminated from the other C-15 atom in the formation of prephytoene pyrophosphate (PPPP) is also the *pro-S* hydrogen, as has been shown in the case of squalene formation [95]. The mechanism proposed would give rise to PPPP with the same chirality as that of presqualene pyrophosphate (1*R*, 2*R*, 3*R*) [134] and the optical properties of PPPP indicate the same chirality [113].

Table 2.1 Natural distribution of all-*trans*- and 15-*cis*-phytoene

Source	Major isomer	Ref.
HIGHER PLANTS		
Carrot (root)	<i>cis</i>	121, 122
Tomato (fruit)	<i>cis</i>	121, 122
FUNGI		
<i>Mucor hiemalis</i> (inhibited)	<i>cis</i>	123
<i>Mucor</i> spp.	<i>cis</i>	124
<i>Neurospora crassa</i>	<i>cis</i>	123
<i>Phycomyces blakesleeanus</i>	<i>cis</i>	121
PHOTOSYNTHETIC BACTERIA		
<i>Rhodomicrobium vannielii</i>	<i>cis:trans</i> (3:1–2:1)	125
<i>Rhodopseudomonas sphaeroides</i> (inhibited)	<i>cis</i>	115
<i>Rhodospirillum rubrum</i> (inhibited cultures)	<i>cis</i>	115, 121
<i>Rhodospirillum rubrum</i> (various mutants)	<i>cis</i>	124
(mutant S <sub>1</sub> B <sub>4</sub> )	<i>trans</i>	124
NON-PHOTOSYNTHETIC BACTERIA		
<i>Flavobacterium</i> ( <i>Cellulomonas</i> ) <i>dehydrogenans</i>	<i>trans</i>	126
<i>Flavobacterium</i> sp. (Roche)	<i>cis</i>	130
<i>Halobacterium cutirubrum</i>	<i>trans</i>	127, 128
<i>Micrococcus</i> sp.	<i>trans</i>	130A
<i>Mycobacterium</i> spp.	<i>trans</i>	101, 129
ALGAE		
<i>Scenedesmus</i> (mutants)	<i>cis</i>	131

#### 2.2.4 DESATURATION OF PHYTOENE

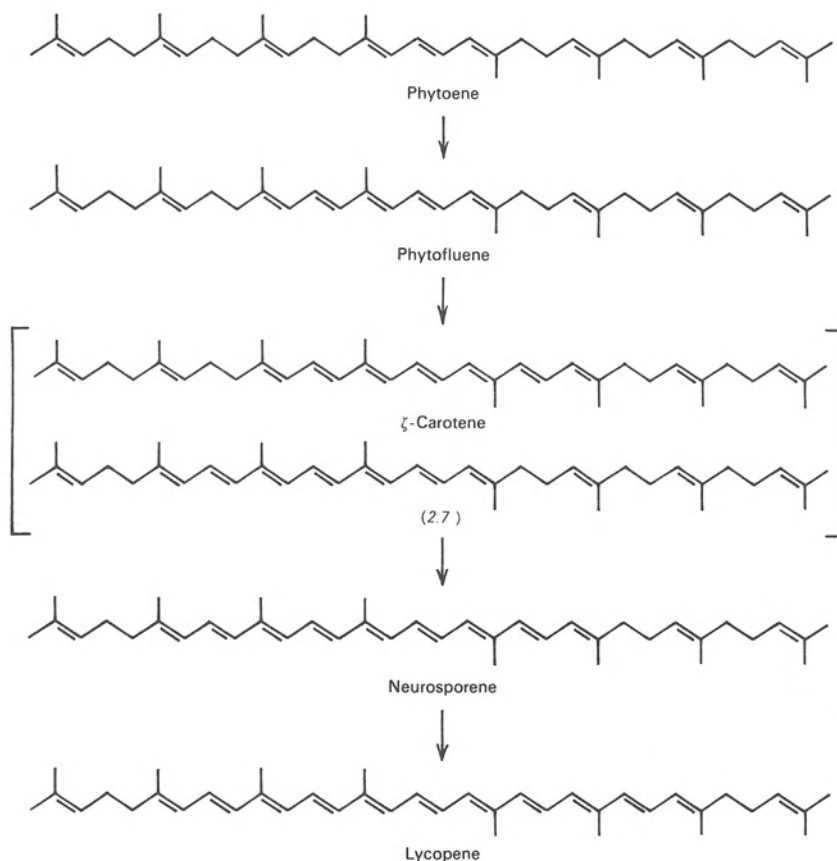
Long before the structures of the intermediates were known the stepwise desaturation of phytoene to form lycopene (1.11) was proposed for tomatoes [135]. The sequence (Fig. 2.13) is now well authenticated. It involves in higher plants and algae the removal of two hydrogens at a time from alternate sides of the molecule to yield, sequentially, phytofluene,  $\zeta$ -carotene, neurosporene and lycopene. In the case of the photosynthetic bacteria *Rhodospirillum rubrum* [136] and *Rhodopseudomonas globiformis* [137]  $\zeta$ -carotene is replaced by its unsymmetrical isomer 7,8,11,12-tetrahydrolycopene (2.7) and its formation involves two successive desaturations on the same side of the molecule. In other photosynthetic bacteria, e.g. *Rhodopseudomonas viridis* [138, 139],



(2.7) 7,8,11,12-Tetrahydrolycopene

non-photosynthetic bacteria (*Cellulomonas* (*Flavobacterium*) *dehydrogenans*) [140, 141] and fungi (*Neurospora crassa* [142] and *P. blakesleeanus* [143]),

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*Fig. 2.13.* The stepwise desaturation of phytoene to form lycopene.

both tetrahydrolycopene isomers exist together; it is not known which is involved in the biosynthetic pathway. It is not inconceivable that both may be involved.

The main bases for concluding that the pathway outlined in Fig. 2.13 represents reality are: (i) mutant studies with many organisms (see the appropriate chapters) indicate these intermediates; (ii) the inhibitor diphenylamine (DPA) causes the accumulation of phytoene at the expense of coloured carotenoids in fungi and bacteria (see Chapters 8, 9, 10); when DPA is washed out of a culture and the culture resuspended in an appropriate medium then the more unsaturated carotenoids are synthesized at the expense of the accumulated intermediates [144–146]; (iii) time-course experiments of synthesis after either removal of an inhibitor, alteration of cultural conditions or incorporation of labelled precursors have been reported which both support [147–150] and oppose [151–155] the pathway outlined above. How-

ever, kinetic experiments are notoriously difficult to interpret unequivocally and full proof will only come with a clear enzymic demonstration of each step. This type of evidence is now accumulating with the aid of purified enzyme preparations from bacteria, fungi, spinach chloroplasts and tomato fruit plastids [128, 130, 156]. The first problem is whether all-*trans*- or 15-*cis*-phytoene is the primary C<sub>40</sub> substrate. The situation probably varies from one system to another and Fig. 2.14 is an attempt to accommodate all available information in one biosynthetic sequence [160].

The strength of experimental evidence in support of each step, however, varies considerably. There is no doubt that both all-*trans*- and 15-*cis*-phytoene can be synthesized by different systems as the *primary* product of dimerization of GGPP: there is also good evidence that *cis*-phytoene can be converted into *trans*-phytoene which can then be sequentially desaturated to form lycopene. There is also some support for the view that the *cis-trans* isomerization can take place at the level of phytofluene and  $\zeta$ -carotene, but although *cis*-phytoene has been converted into *cis*-phytofluene there is no clear evidence for the conversion of this polyene into *cis*- $\zeta$ -carotene (see [184]). The recent demonstration that the polyenes found in tangerine tomatoes are (i) 15-monocis-phytoene, (ii) 15,9'-dicis-phytofluene, (iii) 9,9'-dicis- $\zeta$ -carotene, (iv) 9,7',9'-tricis-neurosporene (proneurosporene) and (v) 7,9,7',9'-tetracislycopene (prolycopene) (1.55) [284] has stimulated a reassessment of the accepted biosynthetic pathway to these compounds. This is discussed further in Chapter 5, but from a structural point of view it seems that phytoene rather than  $\zeta$ -carotene is the branch point for procarotene biosynthesis.

### 2.2.5 NATURE OF ENZYMES INVOLVED IN PHYTOENE DESATURATION

Experimental studies on the *in vitro* conversion of phytoene into lycopene have not yet progressed sufficiently to allow comment with any confidence on the mechanisms involved, but some information has been obtained from genetic studies. Complementation studies with mutants of *Phycomyces blakesleeanus* suggest that the complete desaturation sequence is controlled by the product of one gene (*car R*), with four copies of its product (enzyme) acting in a complex to convert phytoene into lycopene [162, 163]. A leaky *car B* mutant of *P. blakesleeanus* produces large amounts of phytoene and progressively smaller amounts of phytofluene,  $\zeta$ -carotene, neurosporene and lycopene; this suggests that the dehydrogenase complex contains four copies of the same enzyme [164]. However, later studies suggest that such a complex may be only functioning during exponential growth of *P. blakesleeanus* [165].

A similar conclusion about the existence of a dehydrogenase complex arose from genetic studies on *Ustilago violacea* [166].

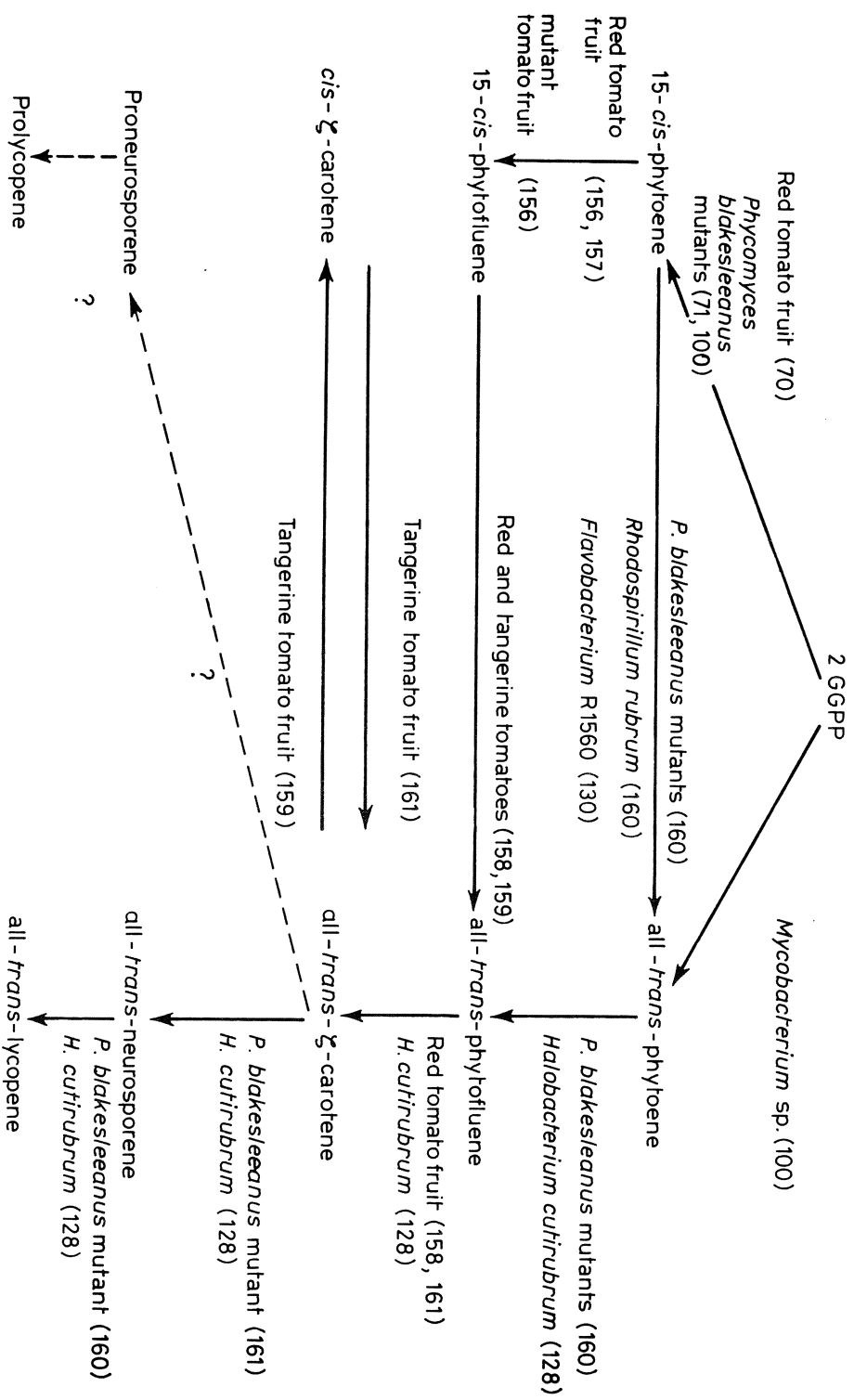


Fig. 2.14. Possible routes of formation of *trans*- $\zeta$ -carotene and *trans*-lycopene and prolycopene from *cis*-phytoene.

## 2.2.6 STEREOCHEMISTRY OF THE DESATURATION

Each step in the desaturation of phytoene produces a double bond and at each step one hydrogen is lost from a carbon atom originating from C-2 of MVA and one is lost from a carbon atom originating from C-5 of MVA. This reaction thus involves the two prochiral centres of MVA at C-2 and C-5. When  $[2-^{14}\text{C}, (5R)-5-^3\text{H}_1]\text{MVA}$  and  $[2-^{14}\text{C}, 5-^3\text{H}_2]\text{MVA}$  were incubated with tomato slices it became clear that the hydrogens lost were those which were originally the 5-*pro-R* hydrogens of MVA [167]. Experiments with  $[2-^{14}\text{C}, (2R)-2-^3\text{H}_1]\text{MVA}$  and  $[2-^{14}\text{C}, (2S)-2-^3\text{H}_1]\text{MVA}$  were much less clear-cut with tomato slices. The reason for this is that the prenyl transferase reaction in higher plants is relatively sluggish [168], which allows significant randomization of label between the 2-*pro-R* and 2-*pro-S* positions of MVA [169] as indicated in Fig. 2.15. However, this difficulty was overcome by preparing GGPP from either  $[2-^{14}\text{C}, (2R)-2-^3\text{H}_1]\text{MVA}$  or  $[2-^{14}\text{C}, (2S)-2-^3\text{H}_1]\text{MVA}$  in an active system from *Marah* (*Echinocystis*) *macrocarpa* endosperm [170]. The resulting carotenes had clearly lost the hydrogens originating from the 2-*pro-S*-hydrogen of MVA [171]. This has now been confirmed in a very efficient carotene-synthesizing system prepared from a *Flavobacterium* sp. [172]. The stereochemical situation of phytoene desaturation is summarized in Fig. 2.16; each step represents a *trans*-elimination of two hydrogen atoms.

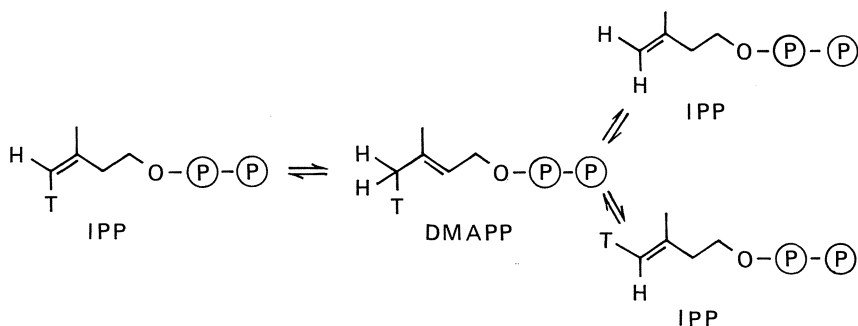


Fig. 2.15. Probable mechanism involved in scrambling the stereospecific label at C-4 of IPP.

## 2.3 FURTHER REACTIONS OF LYCOPENE

Lycopene can undergo a number of metabolic reactions which involve the C-1,2 double bond. These reactions include (i) cyclization, (ii) hydration, (iii) hydrogenation and (iv) addition of a further  $\text{C}_5$  isoprenoid unit with and without cyclization. Each reaction will be considered in detail in turn. Some reactions which do not involve the terminal double bond, e.g. desaturation at C-3,4, will be considered at the end of the section.

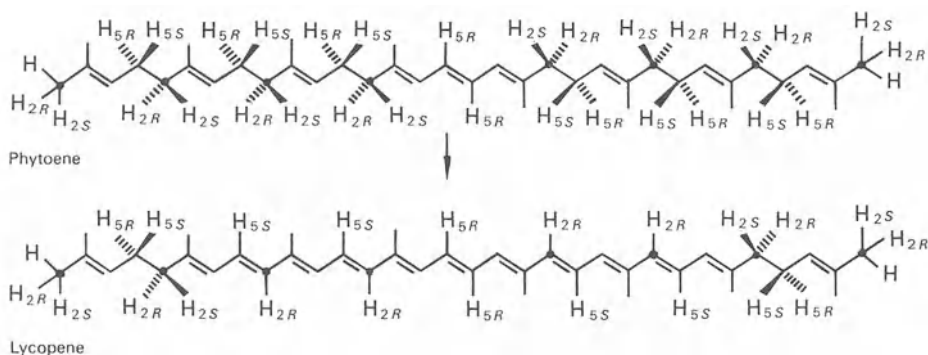


Fig. 2.16. Stereochemistry of desaturation of phytoene in forming lycopene.

### 2.3.1 CYCLIZATION OF LYCOPENE

#### (a) Inhibitor and enzyme studies

Cyclic carotenoids with  $\beta$ - (1.5) and  $\epsilon$ - (1.6) rings are widespread in nature, in particular in photosynthetic organelles of higher plants and algae (see Chapters 4 and 7). A  $\gamma$ -ring (1.7) has so far only been reported in the discomycete *Caloscypha fulgens* [173] (see Chapter 8) and in some insects (see Volume II).

Evidence for lycopene as precursor of cyclic carotenoids includes the following: (i) The conversion of lycopene into cyclic carotenes has been reported in intact chloroplasts [174] and in soluble preparations from spinach chloroplasts [174], tomato plastids [156, 157], *Phycomyces blakesleeanus* [175] and a *Flavobacterium* sp. [176]. (ii) Mutants of *Phycomyces blakesleeanus* [177] have been obtained which accumulate lycopene instead of the  $\beta$ -carotene (1.13) usually found in the wild strain. (iii) In a tomato phenotype carrying the B gene obtained by backcrossing a *Lycopersicon esculentum* and *L. hirsutum* hybrid to *L. esculentum* the normal high level of lycopene is replaced by an equal amount of  $\beta$ -carotene (1.13) [136, 178] and the formation of this  $\beta$ -carotene is temperature-sensitive [178] as is lycopene synthesis in native strains of tomatoes [179]. Furthermore, the fruit of the F<sub>1</sub> generation of this high-B strain contains more  $\gamma$ -carotene (1.38) than do normal strains and  $\gamma$ -carotene is the only possible intermediate between lycopene and  $\beta$ -carotene [180]. (iv) Inhibitors which specifically inhibit cyclization of lycopene in a number of organisms include nicotine [181–190] and the herbicide CPTA [2-(*p*-chlorophenylthio)triethylammonium chloride] [191–204]. They cause the accumulation of lycopene which on removal of the inhibitor is converted into cyclic carotenoids [181–187, 191, 196, 200]. The mechanism involved in the inhibition is not yet known but it appears to be cyclization itself which is blocked rather than the synthesis of the cyclizing enzyme [181,



183, 196]. Other nitrogenous bases in addition to nicotine are effective inhibitors of cyclization (Table 2.2) and attempts have been made to correlate their inhibitory activity with their  $pK_a$  values [205]. Nicotine itself is more effective on *Cucurbita ficifolia* cotyledons under alkaline than under acid conditions [175].

Table 2.2 Some compounds which inhibit cyclization of lycopene

Compound	Organism	Ref.
Imidazole	<i>B. trispora</i>	206
2-Methylimidazole	<i>B. trispora</i>	206
1-Ethyl-2-methylimidazole	<i>B. trispora</i>	206
Pyridine	<i>B. trispora</i>	206, 207
2-Aminopyridine	<i>B. trispora</i>	206
Nicotine	<i>P. blakesleeanus</i>	184, 208
	<i>Flavobacterium</i> R1519 <sup>a</sup>	184
	<i>Verticillium agaricinum</i>	187
	Banana leaves	209
CPTA <sup>b</sup>	<i>P. blakesleeanus</i>	195, 210
	<i>B. trispora</i>	192–196
	Many fruit	192–198
4-[ $\beta$ -(Diethylamino)-ethoxy]-benzaldehyde	<i>B. trispora</i>	203
4-[ $\beta$ -(Diethylamino)-ethoxy]-acetophenone, HCl	<i>B. trispora</i>	203
4-[ $\beta$ -(Diethylamino)-ethoxy]-benzophenone, HCl	<i>B. trispora</i>	203
$\alpha$ -Diethylamino propiophenone HCl	<i>B. trispora</i>	203
Triethylamine HCl	<i>B. trispora</i>	203
Tributylamine HCl	<i>B. trispora</i>	203
Piperidine	<i>B. trispora</i>	211
Cycocel	Pumpkin cotyledons	210
Fluridone	Wheat seedlings	211A
Norflurazon	Wheat seedlings	211A

#### Notes

<sup>a</sup> Both (+) and (–) nicotine are active

<sup>b</sup> 2-(4-Chlorophenylthio)triethylammonium chloride

#### (b) Mechanisms

The accepted mechanism of ring closure (Fig. 2.17) involves firstly proton attack at C-2 with the formation of a 'carbonium ion' intermediate followed by its stabilization by the elimination of one of three different hydrogens to produce the three ring systems. The reality of the proton attack was recently demonstrated with the aid of a mutant of *Scenedesmus obliquus* which when grown in the dark accumulates  $\zeta$ -carotene. When the cells are illuminated the  $\zeta$ -carotene is desaturated to lycopene and cyclized. If the latter step is carried out in D<sub>2</sub>O instead of H<sub>2</sub>O then the resulting cyclic carotenoids contain two atoms of deuterium, located at C-2 and C-2' respectively [212].

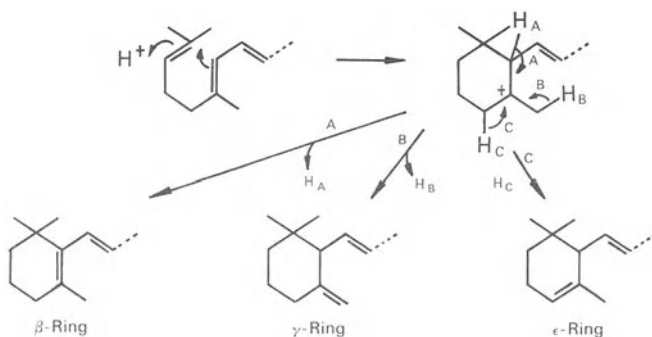


Fig. 2.17. Mechanism of formation of  $\beta$ -,  $\gamma$ -, and  $\epsilon$ -rings from a common carbonium ion intermediate.

The same results have been obtained in *Rhodomicrobium vannielii* (G. Britton *et al.*, unpublished results). Proof of the basic mechanism for the formation of  $\beta$ - and  $\epsilon$ -rings was obtained by many experiments with  $[2\text{-}^{14}\text{C}, (4R)\text{-}4\text{-}^3\text{H}_1]\text{MVA}$  which showed that tritium is lost from C-6 in formation of a  $\beta$ -ring, but is retained in the formation of the  $\epsilon$ -ring (Fig. 2.18a). This indicates the separate formation of the two ring systems because if the  $\epsilon$ -ring

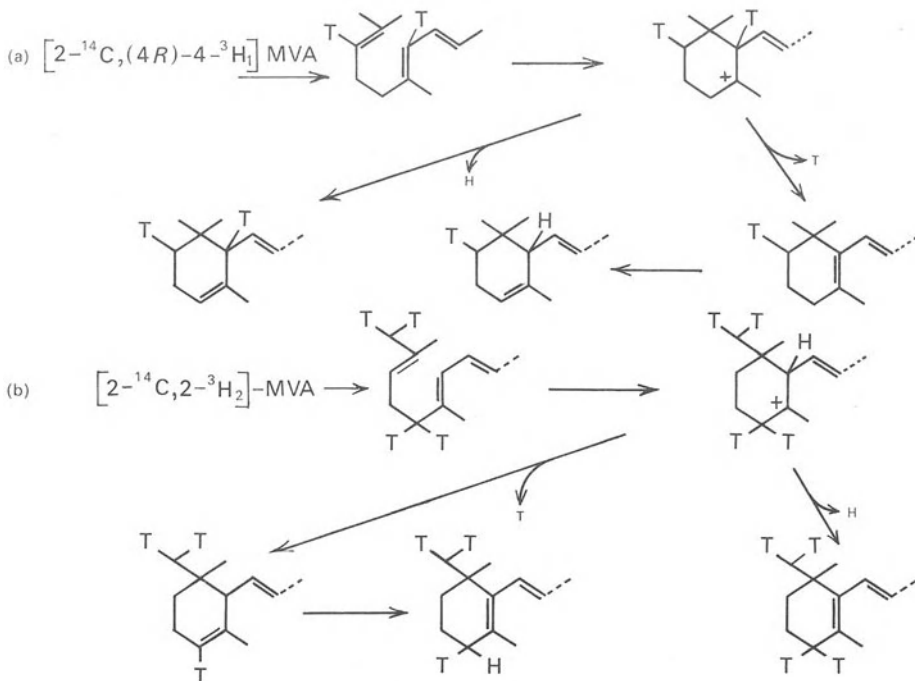


Fig. 2.18. Demonstration of the independent formation of  $\beta$ - and  $\epsilon$ -rings by the use of specifically labelled species of MVA.

were formed from the  $\beta$ -ring then the tritium at C-6 would be lost (Fig. 2.18) [213]. Similar experiments with  $[2-^{14}\text{C}, 2-^3\text{H}_2]\text{MVA}$  demonstrated that the  $\beta$ -ring was not formed by isomerization of the  $\epsilon$ -ring. Fig. 2.18b shows that direct production of the  $\beta$ -ring results in retention of two labelled hydrogen atoms at C-4 whilst isomerization of an  $\epsilon$ -ring would result in only one being present. The experimental results demonstrated retention of both tritiums [213, 214]. Inheritance studies on tomatoes also indicate that the synthesis of the two ring systems is under the control of two different genes [215].

### (c) Formation of $\text{C}_{45}$ and $\text{C}_{50}$ carotenoids

Other cyclic end groups are also encountered in nature, in particular those with an additional  $\text{C}_5$  unit at C-2 and/or at C-2' e.g. decaprenoxanthin (1.29). In the formation of decaprenoxanthin by *Flavobacterium dehydrogenans* the pathway phytoene  $\rightarrow$  lycopene can also be detected [141] so it has been suggested that an electrophilic  $\text{C}_5$  species (e.g. dimethylallyl pyrophosphate) replaces a proton as the initiator of cyclization as indicated in Fig. 2.19.

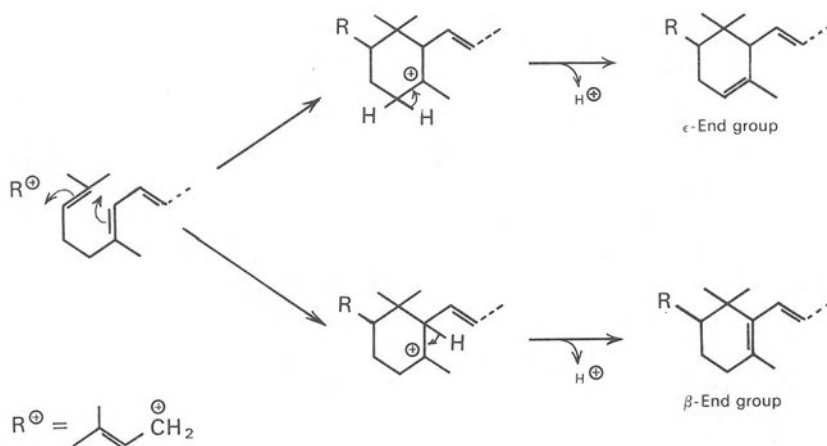


Fig. 2.19. Probable mechanism for the formation of  $\text{C}_{45}$  and  $\text{C}_{50}$  carotenoids with  $\beta$ - and  $\epsilon$ -rings.

### (d) Formation of caroten-2-ols

The formation of the rare caroten-2-ols, pigments found in the alga *Trentepohlia iolithus* [216], can be associated with ring closure in two ways (Fig. 2.20). Firstly by  $\text{HO}^+$  as the initiating species instead of the usual proton or by epoxidation across the 1,2-double bond followed by  $\text{H}^+$ -initiated cyclization. The latter would be the same mechanism as that involved in the conversion of squalene via squalene 1,2-oxide into lanosterol. Such acyclic

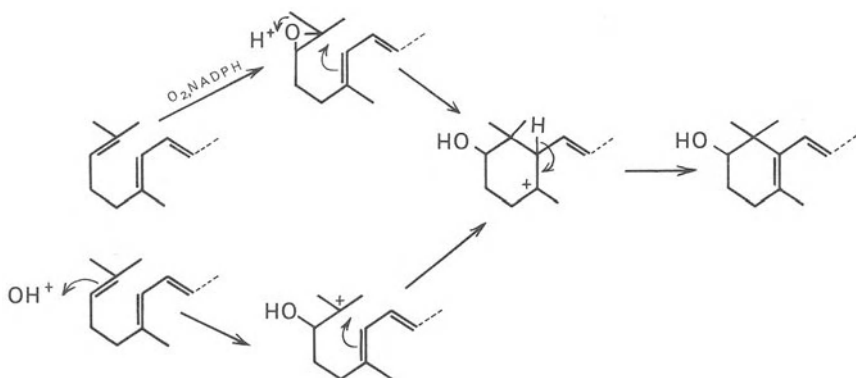
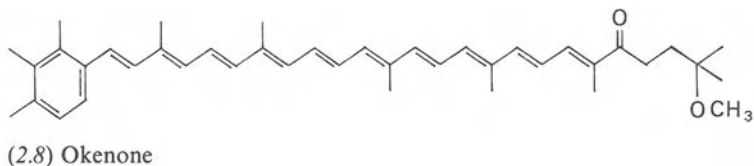


Fig. 2.20. Possible ways of forming caroten-2-ols.

carotenoid epoxides have been found in tomato fruit [217] but it is not known whether or not they occur in *Trentepohlia iolithus*.

#### (e) Formation of aromatic carotenoids

Studies on the biosynthesis of the aromatic ring in chlorobactene (1.53) produced by the green photosynthetic bacterium *Chloropseudomonas ethylica* have not been carried out to demonstrate that the aromatic ring arises from lycopene via the  $\beta$ -ring. However, this is very likely and it has been shown that the aromatic ring is MVA-derived and that the methyl group at C-2 of chlorobactene arises by migration of one of the methyl groups at C-1 of a precursor; the migrating methyl arises specifically from C-3' of MVA whilst that remaining at C-1 arises from C-2 of MVA [218]. In the okenone (2.8) type of aromatic carotenoids synthesized by some photosynthetic bacteria (Chapter 10) the methyl group normally expected to be at C-5 moves to C-3. It has been suggested that a mechanism based on that outlined in Fig. 2.21 is involved in converting the aromatic ring typical of chlorobactene into that found in okenone [219].



#### (f) Carotenoids with cyclopentane rings

The cyclopentane ring characteristic of capsanthin (1.30) and capsorubin (1.59) is found primarily in red peppers and is probably formed from the corresponding epoxide as indicated in Fig. 2.22 [107]. There is yet no experimental evidence to support this view.

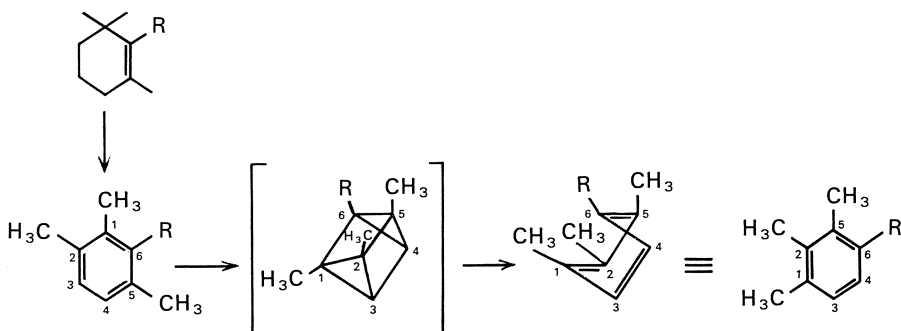


Fig. 2.21. Possible mechanism for synthesizing the two types of aromatic ring found in carotenoids.

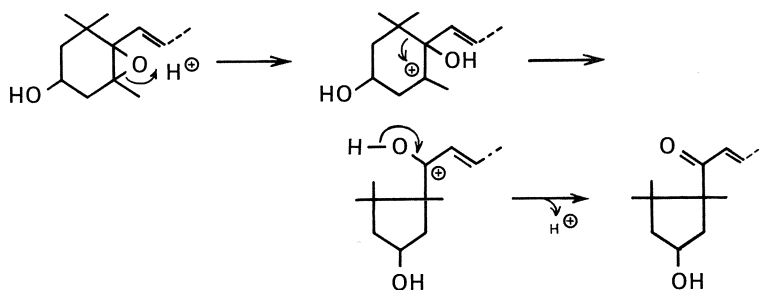
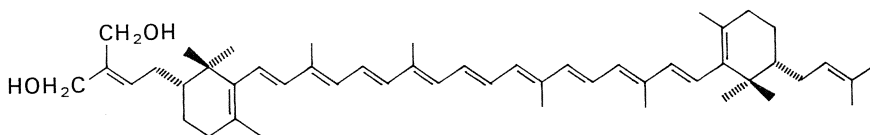


Fig. 2.22. Possible mechanism of formation of cyclopentane rings in carotenoids.

### (g) Stereochemistry of cyclization

In order to understand the mechanisms involved in cyclization the stereochemistry of the reaction must be known. The possibilities envisaged include (i) which conformation of the carbon skeleton is involved; (ii) which faces of the double bonds at C-1,2 and C-5,6 are involved in electrophilic attack and whether the same faces are involved in producing  $\beta$ - and  $\epsilon$ -rings; (iii) what is the orientation of the methyl groups at C-1; (iv) what is the chirality at C-6 in the  $\epsilon$ -ring; and (v) which hydrogen is removed from C-4 in the formation of  $\epsilon$ -rings [220, 221]? Insufficient is known to postulate the conformation involved in cyclization [point (i)]. The possibility of relating the mechanism of proton attack to the stereochemistry at C-2 of the substituted carotenoids was not realized when it was shown that the configuration at C-2 of C.p. 450 (2.9) [222] is opposite to that of the caroten-2-ols [e.g. (1.75)] from *Trentepohlia* [223]. Neither is it possible to relate the stereochemistry of proton attacks in the unsubstituted  $\epsilon$ -ring to decaprenoxanthin (1.29); although the configuration at C-2 is the same as in C.p. 450, the chirality

at C-6' is the opposite [224] to that found in carotenes with unsubstituted  $\epsilon$ -rings, e.g.  $\alpha$ -carotene (1.12) [225, 226]. However, recently it has been shown



(2.9) C.p. 450

that in the formation of the  $\beta$ -ring in zeaxanthin synthesis by a *Flavobacterium* sp. the incoming proton attacks the *re, re* face of the C-1,2 double bond [point (ii)]. This was achieved by growing the organism in the presence of nicotine, which caused the accumulation of lycopene, washing out the inhibitor and resuspending the cells aerobically in  $D_2O$ . The chirality at C-2 which results from the insertion of deuterium at this position in the formation of zeaxanthin was shown by n.m.r. studies to be *S* (Fig. 2.23) [221].

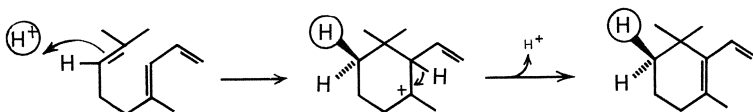


Fig. 2.23. Stereochemistry of hydrogen attack in forming the  $\beta$ -ring of  $\beta$ -carotene and zeaxanthin.

The orientation of the gem methyl groups has recently been defined with the help of  $^{13}C$ -n.m.r. measurements. In zeaxanthin synthesized by a *Flavobacterium* sp. in the presence of  $[2-^{13}C]$ MVA the label was located in the axial 1 $\alpha$ -methyl substituent [227]. Thus the behaviour of the C-1 methyl substituents during cyclization is as indicated in Fig. 2.24, in which the stereochemistry of hydrogen addition at C-2 is also included. The stereochemistry of the reaction at the C-1,2 double bond, equivalent to an overall

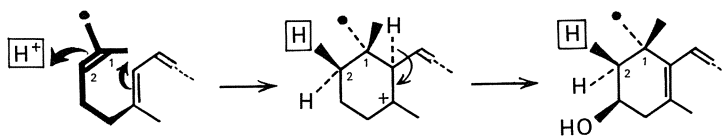
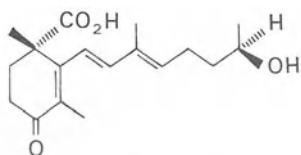
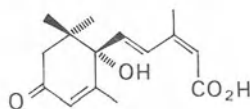


Fig. 2.24. The overall stereochemistry of the formation of zeaxanthin from lycopene in a *Flavobacterium* sp.

*trans* addition, is now defined but the conformation leading to this (chair or boat) remains to be settled. It is interesting that the labelling pattern in the C-1 methyl groups of zeaxanthin in *Flavobacterium* is opposite to that deduced for trisporic acid (2.10) and  $\beta$ -carotene (1.13) in *Blakeslea trispora* [228] and that reported for abscisic acid (2.11) [229].



(2.10) Trisporic acid



(2.11) Abscisis acid

The incorporation of geranylgeranyl pyrophosphate labelled from either  $[2\text{-}^{14}\text{C}, (2R)\text{-}2\text{-}^3\text{H}_1]\text{MVA}$  or  $[2\text{-}^{14}\text{C}, (2S)\text{-}2\text{-}^3\text{H}_1]\text{MVA}$  into  $\alpha$ -carotene by tomato slices has revealed that it is the 2-*pro-S* hydrogen which is lost from C-4 during the formation of the  $\epsilon$ -ring [point (iv)] (Fig. 2.25) [230].

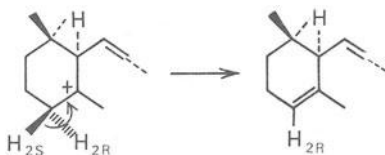
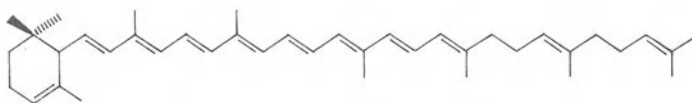
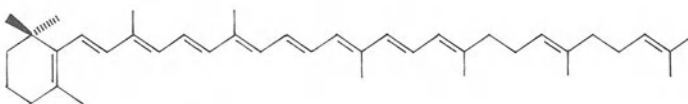
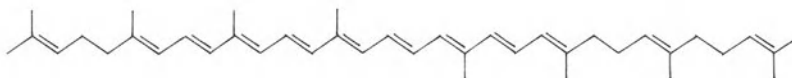


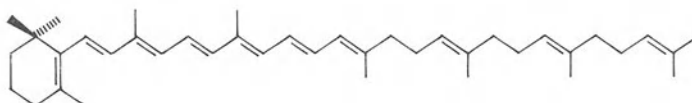
Fig. 2.25. Stereochemistry of hydrogen loss in the formation of the  $\epsilon$ -ring of  $\alpha$ -carotene.

### 2.3.2 CYCLIZATION OF PRECURSORS OTHER THAN LYCOPENE

The existence in nature of  $\alpha$ - and  $\beta$ -zeacarotenes (2.12, 2.13) in maize [231] and tomato mutants (delta) [214] indicates that neurosporene (2.14) can

(2.12)  $\alpha$ -Zeacarotene(2.13)  $\beta$ -Zeacarotene

(2.14) Neurosporene



(2.15) Cyclic isomer of 2.7

cyclize. Furthermore, the absence of a naturally occurring cyclic isomer of  $\zeta$ -carotene (2.15) and the existence of a cyclic isomer ( $\beta$ -ring) of 7,8,11,12-tetrahydro- $\psi,\psi$ -carotene [asymmetrical  $\zeta$ -carotene (2.7)] in DPA-inhibited cultures of *P. blakesleeanus* [143] confirms the conclusion that unsaturation at C-7,8 is a mandatory requirement for cyclization. Thus an overall scheme for the biosynthesis of cyclic carotenoids can be drawn up (Fig. 2.26) but the question arises whether one pathway in the metabolic matrix preponderates to such an extent that it may be the normal pathway *in vivo*. We have assumed that lycopene is the normal precursor of cyclic carotenoids but some evidence for the implication of neurosporene in the normal pathway exists. Tomatoes ripened above 30° C do not synthesize lycopene but continue to make some  $\beta$ -carotene [232, 233] suggesting that lycopene is not a precursor of  $\beta$ -carotene; however, it is more than likely that two distinct and separate sites of synthesis are involved and a mutant which makes massive amounts of  $\beta$ -carotene at the expense of lycopene has already been described (p. 173). A similar compartmentation effect in tomatoes is observed if they are treated with dimethylsulphoxide (DMSO) [234]. Compartmentation can also be invoked to explain the results with the fungus *Rhizophlyctis rosea* which synthesizes lycopene during the early stages of growth and  $\gamma$ -carotene during the later stages. If [2-<sup>14</sup>C]MVA is added after lycopene has reached its peak the  $\gamma$ -carotene becomes strongly labelled whilst the lycopene is only slightly labelled suggesting that lycopene is not a precursor of  $\gamma$ -carotene. Possibly the cyclizing enzyme only develops at a later stage of growth and will only form  $\gamma$ -carotene from newly formed lycopene, the lycopene formed early having been rendered metabolically inert by being transported into storage lipid droplets [235]. [<sup>14</sup>C]-Neurosporene (2.14) has been converted into  $\beta$ -carotene by a preparation from *Phycomyces blakesleeanus* [175]; however, it may well have been converted into lycopene before cyclization occurred.

### 2.3.3 HYDRATION OF LYCOPENE

Carotenoids characteristic of bacteria, particularly photosynthetic bacteria, contain a free or methylated tertiary hydroxyl group at C-1. As the introduction of this hydroxyl group is, in most photosynthetic bacteria at least, an anaerobic process, it is likely that it is a direct addition of water (Fig. 2.27) [220]. The introduction of the hydroxyl group at C-1 is inhibited by nicotine

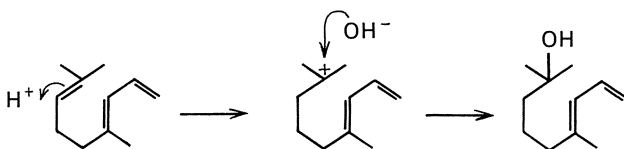
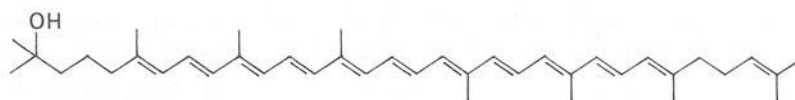


Fig. 2.27. Mechanism for addition of water across the C-1,2 double bond.



## 60 The biochemistry of the carotenoids

and CPTA in *Rhodomicrobium vannielii* [182, 236–238] so that the major carotenoid rhodopin (2.16) is replaced by lycopene; removal of the inhibitor then allows hydration to proceed and rhodopin accumulates as lycopene disappears. Furthermore, if hydration is allowed to proceed in the presence of D<sub>2</sub>O, one deuterium atom appears in the rhodopin molecule (G. Britton *et al.*, unpublished observations). *Rm. vannielii*, rather exceptionally, produces  $\beta$ -carotene (1.13) and the formation of this is also inhibited by nicotine but at a lower concentration than that required for inhibition of hydration [238]. This is also true of *Myxococcus fulvus* [186, 189]. Hydration is also inhibited by nicotine and CPTA in *Rhodospirillum rubrum* in which lycopene accumulates in place of spirilloxanthin (1.19) and in *Rhodopseudomonas sphaeroides* in which neurosporene (2.14) accumulates in place of spheroidene (1.41) [238, 239]. These results suggest that the 7,8-double bond must appear in the molecule before hydration occurs, as is the case with cyclization, but in

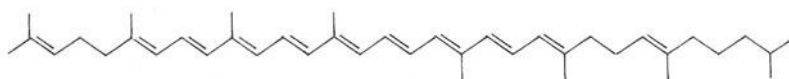


(2.16) Rhodopin

fact hydroxyspheroidene appears in *Rps. sphaeroides* [221] and in the presence of DPA *Rsp. rubrum* produces small amounts of hydrated derivatives of phytoene (1.36), phytofluene (1.37) and 7,8,11,12-tetrahydrolycopene (2.7) [240–244]. These occur only under stress of inhibitors and have not been observed in cultures growing naturally. Enzymic studies have demonstrated the conversion of lycopene into rhodopin in a cell-free preparation from *Rm. vannielii* [238].

### 2.3.4 HYDROGENATION OF LYCOPENE

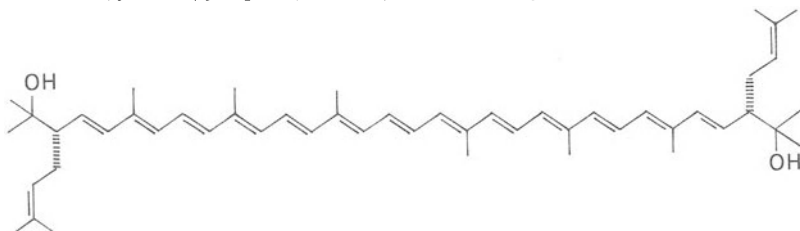
Hydrogenation of the C-1,2 double bond of an acyclic carotene is only known to occur in one species of photosynthetic bacterium, *Rhodopseudomonas viridis* [138, 139]. The main pigment is 1,2-dihydroneurosporene (2.17) in which the more saturated end of the molecule is hydrogenated; that is, desaturation at C-7,8 is not a pre-requisite for hydrogenation at C-1,2 as it is for cyclization and for hydration in normal cultures. As with the last two reactions discussed, hydrogenation is inhibited by CPTA but, in contrast, not appreciably by nicotine [239].



(2.17) 1,2-Dihydroneurosporene

2.3.5 ADDITION OF  $C_5$  UNITS TO  $C_{40}$  UNITS WITHOUT CYCLIZATION

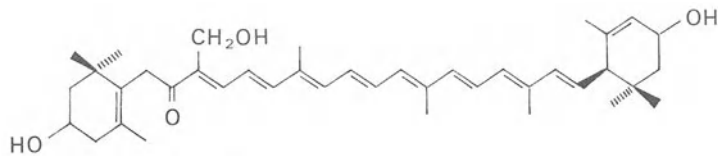
The biosynthesis of bacterioruberin (1.77) and related compounds in *Halo-bacterium salinarum* [245, 246] has not yet been studied in detail but in the presence of nicotine lycopen and bisanhydrobacterioruberin (2.18) accumulate; on removal of the inhibitor both these compounds disappear and bacterioruberin appears. This suggests that  $C_5$  units are added to a conventional  $C_{40}$  unit (lycopene) [190] (see also Chapter 10).



(2.18) Bisanhydrobacterioruberin

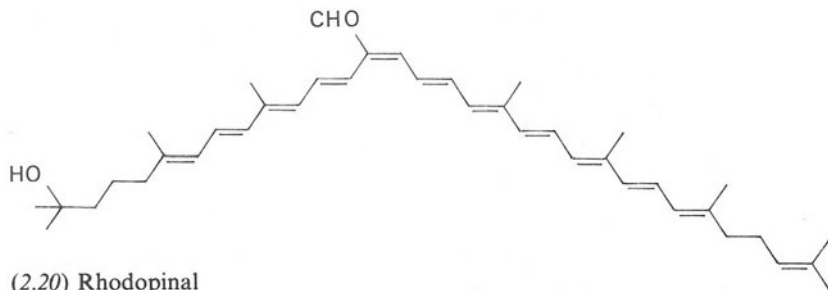
## 2.4 INSERTION OF OXYGEN INTO CAROTENOIDS

The insertion of oxygen at C-1 and C-2 has already been discussed in relation to hydration of the C-1,2 double bond (p. 59) and cyclization reactions (p. 54). Hydroxyl groups are also inserted into the molecule at C-3 as, for example, in zeaxanthin (3,3'-dihydroxy- $\beta$ -carotene), at C-4 as in isocryptoxanthin (1.35), and at C-19 as in siphonaxanthin (2.19). There also



(2.19) Siphonaxanthin

occur 5,6-epoxy carotenoids such as antheraxanthin (1.24) and 5,8-epoxycarotenoids such as mutatochrome (1.44), as well as aldehyde and keto derivatives, e.g. rhodopinal (2.20) and echinenone (1.22) respectively. Finally carotenoid carboxylic acids, such as torularhodin (1.21) are found occasionally. The formation of these xanthophylls will be considered separately.



(2.20) Rhodopinal

## 2.4.1 HYDROXYLATION

Hydroxylation of carotenoids at C-3 has been studied most thoroughly. The first indications that in the formation of 3-hydroxy cyclic carotenoids hydroxylation occurs after cyclization came from studies with some *Chlorella* mutants which, when grown heterotrophically in the dark synthesize only phytoene and  $\zeta$ -carotene. On illumination in the absence of oxygen carotenes are formed which are apparently converted into xanthophylls when oxygen is allowed into the system [247–250]. Kinetic studies with native *Chlorella* also indicates the conversion of  $\alpha$ - and  $\beta$ -carotenes into xanthophylls [251]. More recent work with a *Flavobacterium* sp. has confirmed and extended these studies [182, 184, 185]. This organism normally produces large amounts of zeaxanthin but, in the presence of nicotine, lycopene accumulates in place of zeaxanthin; if hydroxylation occurred before cyclization then hydroxyl-lycopene and not lycopene would have been expected to appear. Furthermore, if the inhibitor is removed and the cells are resuspended anaerobically  $\beta$ -carotene accumulates; if oxygen is subsequently bubbled through the culture the  $\beta$ -carotene is converted into zeaxanthin. If the washed anaerobic cells are resuspended aerobically in  $D_2O$  then the resulting zeaxanthin contains only two deuteriums which are located at C-2 and C-2' [252]. This proves conclusively that the zeaxanthin formed arises from the lycopene which accumulated in the presence of nicotine. The absolute configuration of zeaxanthin is  $3R,3'R$  (2.18) and experiments with  $[2-^{14}C, (5R)-5-^3H_1]MVA$  and  $[2-^{14}C, 5-^3H_2]MVA$  have shown that there is only one hydrogen lost during hydroxylation and that which is lost was originally the 5-*pro-R*-hydrogen of MVA (Fig. 2.28). Thus hydroxylation occurs with retention of configuration [253], a situation which suggests that the reaction is mediated by a mixed function oxidase. The same stereochemistry was observed with

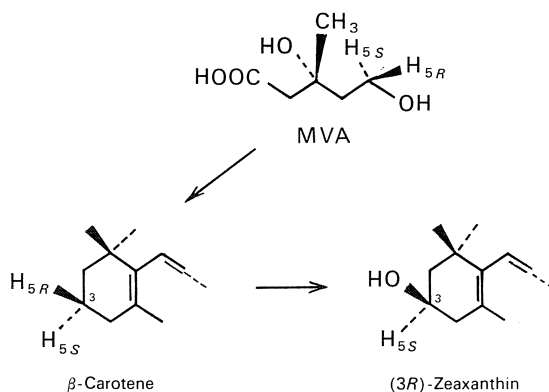


Fig. 2.28. Stereochemistry of reaction involving hydroxylation of  $\beta$ -carotene at C-3 to form zeaxanthin.

zeaxanthin synthesized by the fruit of *Physalis alkekengi* [254]. Although the absolute configuration of lutein at C-3' is *R*, that is opposite to that of zeaxanthin [255], the stereochemistry of the enzymic reaction is, according to preliminary results, the same as that in zeaxanthin [256]. The significance of this remains to be explained.

The oxygen of the hydroxyl group at C-3 in xanthophylls of higher plants arises only from molecular oxygen [257] which also suggests the action of a mixed function oxidase. One objection to the conclusion that the mechanism always involves a mixed function oxygenase is the discovery of  $\beta$ -cryptoxanthin (1.60) in the strict anaerobe *Rhodomicrobium vannielii* [258].

Hydroxylation at C-2 may be associated with ring formation (see p. 54) but hydroxylation at C-4 and at lateral methyl groups (C-19) has not yet been studied.

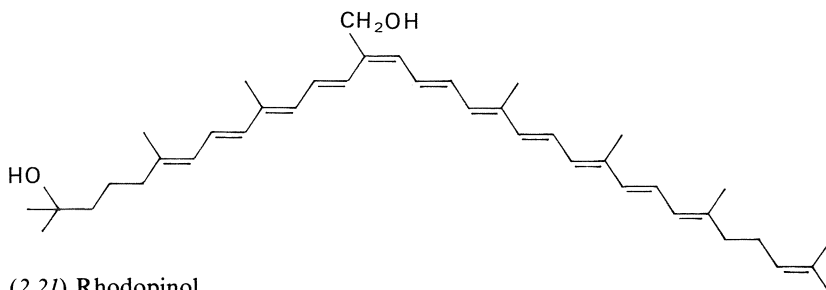
#### 2.4.2 METHYLATION OF HYDROXYL GROUPS

Methylation of the hydroxyl group at C-1 is widespread amongst the photosynthetic bacteria (see Chapter 10) and appears to be unique to this group of organisms. The methyl group arises conventionally [259, 260] from *S*-adenosylmethionine [261].

#### 2.4.3 INSERTION OF CARBONYL GROUPS

A number of photosynthetic bacteria produce carotenoids with carbonyl groups, the best known being spheroidenone (1.42) with the oxygen at C-2. This is formed from spheroidene (1.41) which accumulates in anaerobic cultures of *Rhodopseudomonas sphaeroides* [262, 263]; on exposure to oxygen this compound is converted into spheroidenone and the oxygen at C-2 arises from  $O_2$  and not from  $H_2O$  [264]. A double bond at C-3 appears to be necessary for the introduction of oxygen.

The introduction of a carbonyl function at C-20 in rhodopinal (2.20) in *Chromatium warmingii* [265, 266] and at C-4 in okenone (2.8) in *C. okenii* [267] and related compounds in *Rhodopseudomonas globiformis* [268] occurs under strictly anaerobic conditions and therefore cannot involve  $O_2$ . Rhodo-



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pinol (2.21), a possible intermediate in the reaction leading to rhodopinol (2.20) has been detected in traces in *C. warmingii* [268] but no biochemical evidence for its role as an intermediate has yet been reported and no mechanism has been proposed although the introduction of a double bond may be a first step. In the case of okenone formation a double bond is already present in the appropriate position and hydration of the double bond followed by dehydrogenation would yield the ketone as indicated in Fig. 2.29. Cyclic carotenoids also occur with a carbonyl function at C-4. Inhibitor studies

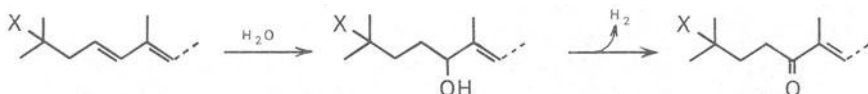


Fig. 2.29. Mechanism for the introduction anaerobically of a carbonyl group at C-4 in okenone and related compounds in photosynthetic bacteria.

(DPA) in the alga *Dictyococcus cinnabarinus* suggest that the corresponding alcohols, isocryptoxanthin (4-hydroxy- $\beta$ -carotene) and isozeaxanthin (4,4'-dihydroxy- $\beta$ -carotene) are precursors of echinenone (4-keto- $\beta$ -carotene) (1.22) and canthaxanthin (4,4'-diketo- $\beta$ -carotene), respectively [269]. Similar results were obtained from the study of glutathione inhibition of a strain of *Brevibacterium* [191].

### 2.4.4 FORMATION OF EPOXIDES

Carotenoid 5,6-epoxides are widespread in nature. It has been known for some time that the source of the incoming oxygen was molecular oxygen [270] and not water, as originally thought [271, 272], but only recently has the basic reaction in their formation been revealed. The conversion is mediated by a mixed function oxygenase which requires NADPH and  $O_2$  as co-factors; a possible mechanism for the reaction is outlined in Fig. 2.30. In lettuce chloroplasts which carry the reaction through from zeaxanthin (2.18)

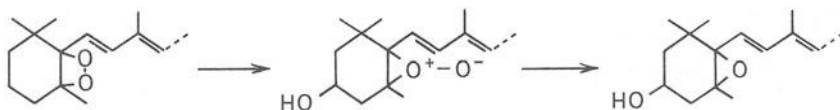
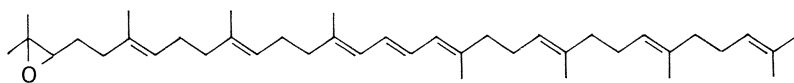


Fig. 2.30. Postulated mechanism for the formation of 5,6-epoxides.

to violaxanthin (1.58) via antheraxanthin (1.24) the optimum pH in the light is 7.8 and in the dark 7.4 [273]. Acyclic 1,2-epoxides [e.g. phytoene epoxide (2.22)] have been obtained from tomato fruit [217, 274]; it would appear that they are not concerned with the cyclization process except possibly in the synthesis of  $\beta$ -rings substituted with a hydroxyl group at C-2 as discussed on p. 54. The presence of traces of carotene 1,2-epoxides in tomatoes may be due to a non-specific action of squalene oxidase.



(2.22) Phytoene epoxide

5,8-Epoxydes (e.g. mutatochrome (1.44) also occur in nature. They are formed very rapidly from 5,6-epoxides under slightly acid conditions and many reported 5,8-epoxydes may, for this reason, be artefacts.

#### 2.4.5 SECOCAROTENOIDS

Secocarotenoids such as semi- $\beta$ -carotenone (2.23) found in citrus fruit, can be formed chemically by partial oxidation of the parent carotenoid. It has been suggested that biologically this is brought about by rearrangement of a hypothetical peroxide of  $\beta$ -carotene (Fig. 2.31) [275].

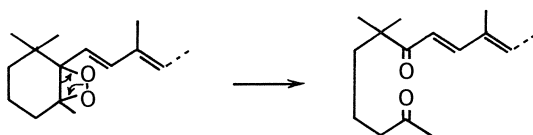
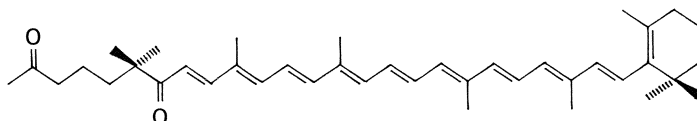
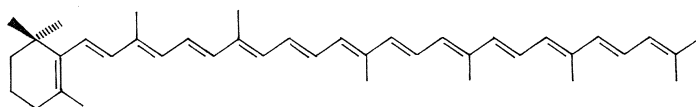


Fig. 2.31. Possible mechanism for the formation of seco-carotenoids.

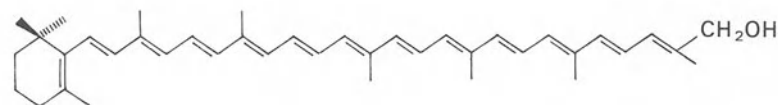
(2.23) Semi- $\beta$ -carotenone

#### 2.4.6 CAROTENOID ACIDS

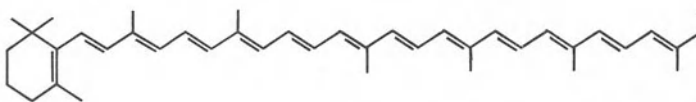
One of the characteristic pigments of red yeasts is torularhodin (1.21) (3',4'-didehydro- $\beta,\psi$ -caroten-16'-oic acid). As it occurs together with torulene (2.24), torularhodin alcohol (2.25) and torularhodin aldehyde (1.23) [276] the obvious biosynthetic pathway (Fig. 2.32) for its formation has been proposed [277, 278]). The only experimental support for this reasonable view is that one oxygen atom from one molecule of molecular oxygen is incorpor-



(2.24) Torulene

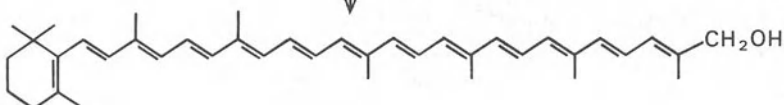


(2.25) Torularhodin alcohol



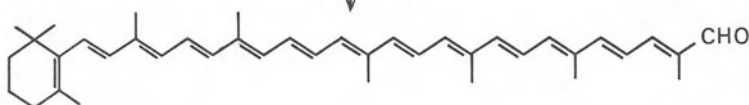
Torulene

↓  
Oxidation



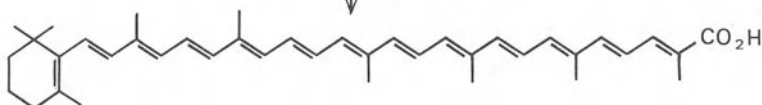
Torularhodin alcohol

↓  
Oxidation



Torularhodinaldehyde

↓  
Oxidation



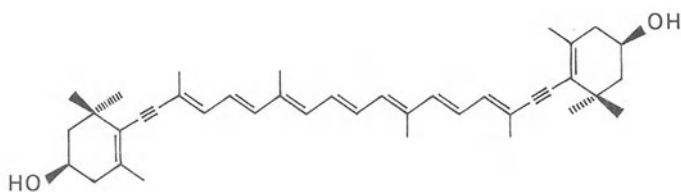
Torularhodin

Fig. 2.32. Probable pathway of torularhodin biosynthesis from torulene.

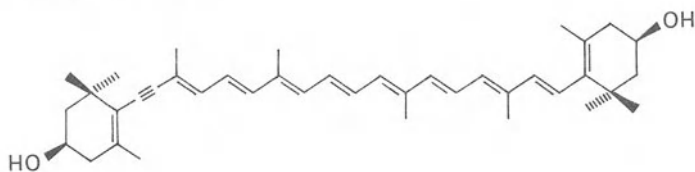
ated into the carboxyl group. This indicates that the first step is hydroxylation of the methyl group at C-16 by a mixed function oxidase [278]. Experiments with  $[2-^{14}\text{C}, 2-^3\text{H}_2]\text{MVA}$  show that the carboxyl group arises specifically from C-2 of MVA, that is the geminal methyl groups at C-1 retain their individuality during the oxidation [279].

## 2.5 ACETYLENIC, ALLENIC AND RETROCAROTENOIDS

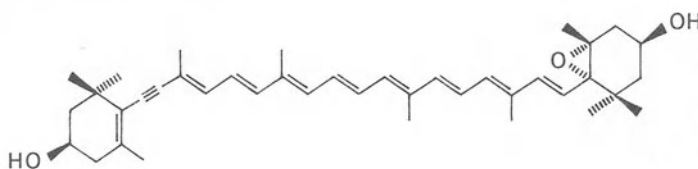
A number of algae produce carotenoids in which an acetylenic group appears at C-7,8; an example of such a pigment is alloxanthin (2.26). Similarly allenes such as neoxanthin (1.47) and fucoxanthin (1.57) are widespread in higher plant leaves and in brown algae, respectively. These structures clearly suggest a close biosynthetic link between acetylenes and allenes and fucoxan-



(2.26) Alloxanthin



(2.27) Diatoxanthin



(2.28) Diadinoxanthin

thin and the acetylenic diatoxanthin (2.27) are found together in some diatoms [280] and neoxanthin and the acetylene diadinoxanthin (2.28) coexist in *Euglena* [281, 282]. An allene could be formed from an epoxide (Fig. 2.33)

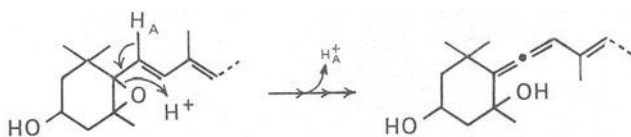


Fig. 2.33. Possible mechanism for allene formation.

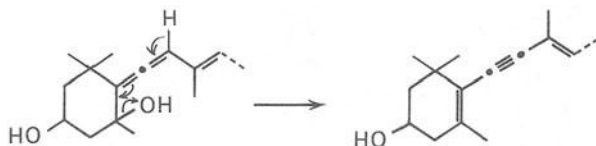


Fig. 2.34. Possible mechanism for isomerization of an allenic carotenoid into an acetylenic carotenoid.

and the acetylene from an allene (Fig. 2.34) and this latter conversion is supported by their co-occurrence in nature just quoted. The failure to find any acetylenic carotenoids in the leaves of higher plants, where neoxanthin is ubiquitous may mean that the required enzyme which used neoxanthin as



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substrate for acetylene formation is missing. The same situation possibly obtains in *Fucus vesiculosus* [281] where the massive amounts of fucoxanthin present are not accompanied by any acetylenic derivatives.

The biosynthesis of the *retro*-carotenoid eschscholtzxanthin (1.25) from *Eschscholtzia californica* has been studied using  $[2-^{14}\text{C},(4R)\text{-}4\text{-}^3\text{H}_1]\text{MVA}$  [283]. The results are consistent with its formation from antheraxanthin (1.24) as indicated in Fig. 2.35.

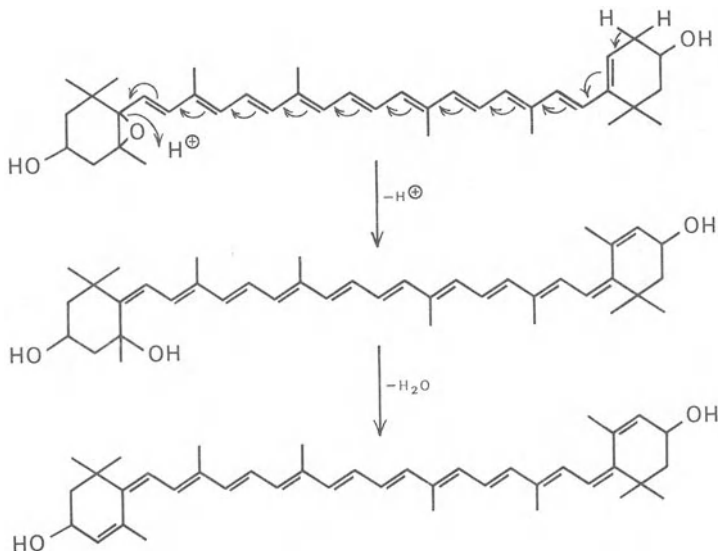


Fig. 2.35. Probable mechanism for the formation of eschscholtzxanthin from antheraxanthin.

## 2.6 C<sub>30</sub> CAROTENOIDS

The formation of these newly discovered pigments, which appear to be confined to certain non-photosynthetic bacteria, is fully discussed in Chapter 9.

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## FUNCTIONS OF CAROTENOIDS

## 3.1 INTRODUCTION

The established functions of carotenoids in plants can all be related to their ability to absorb visible light. In the case of photosynthetic tissues they appear to have two well defined functions: (a) in photosynthesis itself and (b) in protection of the photosynthetic tissue against photosensitized oxidation. In non-photosynthetic tissues of higher plants and in fungi and non-photosynthetic bacteria, carotenoids also take part in photoprotection but the mechanism appears to be different from that in photosynthetic tissues.

Many other functional possibilities have been suggested over the years and the less bizarre ones will be dealt with at the end of the appropriate chapter. The major part of the present discussion will, however, be concerned with photosynthesis and photoprotection.

## 3.2 PHOTOSYNTHESIS

## 3.2.1 ENERGY TRANSFER

(a) *Light harvesting*

The action spectrum for photosynthesis is measured by plotting the quantum yield for photosynthesis against wavelength of the exciting light. Such spectra indicate that light not absorbed by chlorophylls is also photosynthetically active (Fig. 3.1). The first experiments which indicated that light absorbed by carotenoids was utilized for photosynthesis were carried out by Warburg and Negelein [1] with *Chlorella*. They showed that carotenoid-absorbed light was used at low efficiency. Later this was confirmed for other green algae, (*Ulva* spp.) [2-5], and for higher plants [4-6] and the efficiency of transfer was about 50%. However, with brown algae [2, 7] and diatoms [8, 9] (*Nitzschia* spp.) which contain the specific carotenoid fucoxanthin (1.57) the efficiency of the carotenoid-absorbed light was almost as high as that of

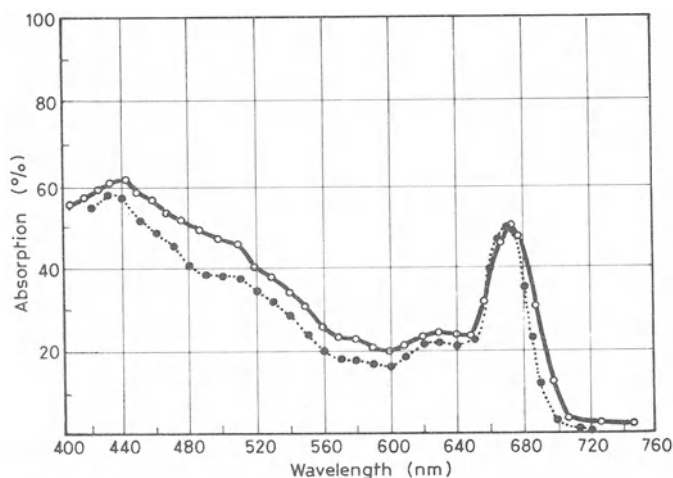


Fig. 3.1. A comparison of the action spectrum (●.....●) for photosynthesis with the absorption spectrum (○-○) of the thallus in the brown alga *Coilodesme*. It will be seen that the two curves closely parallel each other in the region of the spectrum (450–520 nm) where chlorophyll absorption is minimal and carotenoid absorption is maximal. (Redrawn from [3].)

chlorophyll-absorbed light itself. In contrast, the efficiency of carotenoid-absorbed light for photosynthesis in red algae [3] and blue-green algae [2, 4, 5, 10] is very low.

A similar response to carotenoid-absorbed light can be elicited in the photosynthetic bacteria *Rhodospseudomonas sphaeroides* and *Rhodospirillum rubrum* with an efficiency of 90% and 25% respectively [11]. Carotenoid-absorbed light is also involved in the photoassimilation of CO<sub>2</sub> by *Chromatium* sp. [12, 13]; this may not involve the normal pathway of photosynthesis in the organism.

A rather more convenient way of measuring energy transfer from carotenoids and chlorophylls in higher plants and algae is to measure the enhancement of fluorescence of chlorophyll on illumination with light of wavelength absorbed by carotenoids. This technique showed the effective participation of fucoxanthin (1.57) in photosynthesis in diatoms [10, 14] and allowed more detailed investigations to be carried out on greening etiolated seedlings. Results indicated that after 15 minutes illumination of etiolated seedlings carotenoids were not functional in photosynthesis but that very efficient transfer of carotenoid-absorbed light to chlorophyll occurred after four hours [15–20]. An explanation of this is that the functional carotenoid is  $\beta$ -carotene (1.13) and not any of the xanthophylls present. As discussed in Chapter 4, etiolated seedlings contain xanthophylls but little, if any,  $\beta$ -carotene which only appears in significant amounts some time after illumination. Furthermore extraction of leaves with light petroleum, which

removes  $\beta$ -carotene rather than xanthophylls, eliminates the transfer of carotenoid-absorbed light to chlorophyll [21, 22].

In higher plants and algae photosynthesis involves two systems, photosystem I and photosystem II, each of which carries out an essential step in the overall production of reducing power and ATP from the light energy which they absorb. This process is illustrated simply in Fig. 3.2. These systems can be isolated and purified and there is a differential distribution of carotenoids between them. Carotenes tend to accumulate in photosystem I

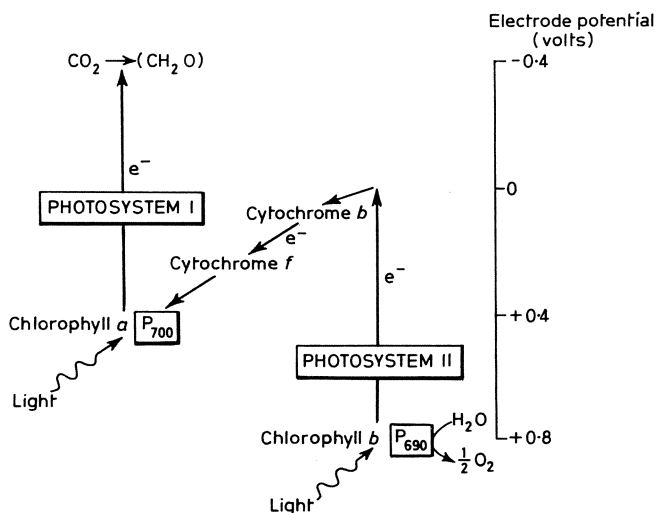
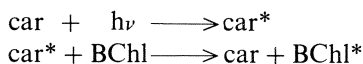


Fig. 3.2. Outline scheme indicating the two photosystems concerned with photosynthesis in aerobic organisms.

and xanthophylls in photosystem II (see Chapter 4). The question is whether carotenoids function in both these systems. The fact that chlorophyll fluorescence is associated with photosystem II suggests that the light-gathering and energy functions of carotenoids are associated with the functions of photosystem II. Experiments with freeze-dried chloroplasts support this view because they show that  $\beta$ -carotene, together with plastoquinone, is necessary for the photosystem II-linked oxidation of cytochrome b-559<sub>HP</sub> [23]. This is in spite of there being more  $\beta$ -carotene in photosystem I than in photosystem II. Work with phycobilin-free lamellae from blue-green and red algae, however, demonstrated that energy transfer from  $\beta$ -carotene to the chlorophyll of photosystem I is very efficient and although xanthophylls are present in the lamellar fraction which contains photosystem II, no energy transfer occurs from these pigments to chlorophylls [24]. This discrepancy remains to be resolved but the latter experiments may not directly mirror the reactions *in vivo*.

In the light harvesting role of carotenoids it seems that it is singlet-singlet energy transfer to bacteriochlorophyll which is involved thus:



### (b) Fluorescence quenching

Light absorbed by carotenoids in some photosynthetic bacteria quenches the fluorescence of bacteriochlorophyll. This phenomenon was originally described in the photosynthetic bacterium *Chromatium* spp. [25–27] and is much smaller in *Rps. sphaeroides* [26] than in *Rsp. rubrum* [26] or *Rsp. molischianum* [28]. In *Rps. sphaeroides* and *Rsp. rubrum* the results are opposite to those expected; that is, carotenoid-absorbed light is more effective in quenching fluorescence in *Rsp. rubrum* than in *Rps. sphaeroides*, although, as indicated in the previous section, the opposite situation obtains for photosynthesis. This suggests that two different carotenoids are involved in the two phenomena. It may be that the pigment actively involved in fluorescence quenching is spirilloxanthin (1.9); it is the major carotenoid in mature *Rsp. rubrum* but a minor component in *Rps. sphaeroides* and *Rsp. molischianum* in which the efficiency of fluorescence quenching by carotenoid-absorbed light is low [26] (see Chapter 10 for a full description of the pigments in these bacteria). The latest work on the photoprotective action of carotenoids involves this phenomenon (Section 3.3).

### 3.2.2 REACTION CENTRE CAROTENOIDS

Some twenty years ago it was shown that illumination of *Chlorella* cells resulted in a decrease in absorbance at 478 nm and an increase at 515 nm [29]. A similar change was observed in *Rsp. rubrum* when an anaerobic culture was illuminated [30] or oxygenated [31]. When the *Chlorella* effect was confirmed in native strains of the alga but was absent in a mutant of *Chlamydomonas reinhardtii*, which lacked  $\beta$ -carotene [32, 33], it was concluded that a carotenoid was involved. The change also does not occur in carotenoid-less mutants of *Rps. sphaeroides* [34]. The same conclusions were reached for the red alga *Iridoea* [35], the xanthophyte *Botrydiopsis alpina* [36], as well as many photosynthetic bacteria [37] including *Rps. sphaeroides* [38, 39], *Rps. capsulata* [40, 41] and *Rps. gelatinosa* [42]. The type of change observed is indicated in Fig. 3.3. The shift has also been observed at  $-35^\circ\text{C}$  to  $-50^\circ\text{C}$  in isolated spinach chloroplasts [43]. In a barley mutant lacking chlorophyll *b*, illumination produces a change in the absorption spectrum of the chloroplast which can also be attributed to a carotenoid shift [44]. The shift is associated with the photosystem I carotenoids [45] and the activity of photosystem I can be reduced by removal of  $\beta$ -carotene and restored by adding it back; xanthophylls are not active [45A].

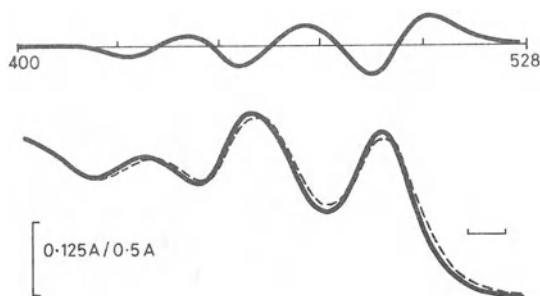


Fig. 3.3. Light-induced carotenoid shift in chromatophores of mutant GIC of *Rhodospseudomonas sphaeroides*. *Top*: light-dark difference spectrum. *Bottom*: absorption spectrum in dark (—) and light (-----). The use of a mutant which contains essentially one carotenoid [neurosporene (2.14)] simplifies the situation and the 'carotenoid shift' is clearly demonstrated [42].

Most of the later work on the carotenoid shift, which is associated with the triplet state of the reaction centre carotenoid [46], has been carried out on the photosynthetic bacteria and a general picture of the present position is outlined here. However, many problems remain. The shift is related to the high energy state of the chromatophores [47, 48] for it is inhibited in isolated chromatophores from *Rps. sphaeroides* by uncoupling agents, by valincomycin and by inhibitors of electron flow [38]. The high quantum efficiency [3] of the change excludes a chemical reaction and it would appear that the shift originates from a charge separation across the photosynthetic membrane; this would represent an electrochromic effect, that is, an absorption change of the bulk pigments lying in the membrane, as demonstrated in chloroplasts [49]. A potential difference induced across the photosynthetic membrane by ionic gradients operating through ionophores gives rise to carotenoid band shifts [50, 51]. The shift also depends in linear fashion on the redox potential which, in *Rps. sphaeroides* chromatophores under steady state conditions, is 210 mV [49].

The shift can probably be separated into three stages: the first which occurs within the picosecond ( $10^{-12}$ ) range, involves more than 20% of the carotenoid molecules and implies that they are bound closely to, if not directly to, the reaction centre; this stage is probably associated with the formation of a transient excited state ( $P^F$ ) of bacteriochlorophyll [52], the decay of which is coupled directly to the formation of  $^3\text{car}$ , the excited triplet state of carotenoids.\* The triplet state of carotenoids has been detected both *in vitro*

\* The ground state of a molecule is known as the singlet  $S_0$  state in which each molecular orbital when full has two electrons with opposite spins; that is, the spins are paired. Absorption of light energy will raise the molecule to the *first excited state*, singlet  $S_1$ , in which the two electrons are in different orbitals but with their spins still paired. The life time of the  $S_1$  state is very short (about  $10^{-9}$  s) and in returning to the ground state a photon is emitted as fluorescence. A molecule in the  $S_1$  state can, however, be converted by collision with other

[53] and in *Chlorella* [54] and spinach chloroplasts [54A], using pulse radiolysis and laser flash photolysis, and in chromatophores of *Rps. sphaeroides* subject to short supersaturating flashes [55–57].

The efficiency of coupling between  $P^F$  and car is almost 100% [58–60]. These results have been obtained with *Rps. sphaeroides* [58–60], *Rsp. rubrum* [61] and *Chromatium vinosum* [62]. In carotenoid-free mutants  $P^F$  decays to yield a longer lived state which is probably the triplet state ( $^3BChl$ ) of bacteriochlorophyll [55, 63].  $^3BChl$  is not detectable in wild-type strains so these experiments clearly implicate carotenoids and their triplet excited states in the primary act of photosynthesis. This fast phase is probably associated with protection against photodynamic sensitization (see Section 3.3.1).

The slower second phase (half-life 0.002–0.20 ms) may be a response to photo-oxidation of cytochrome *c* and a simultaneous reduction of a charged chlorophyll species. The third and slowest phase (half-life 0.02–0.20 ms) is probably concerned with the reduction of cytochrome  $b_{551}$ . Thus all these events are connected with pulsed electron transfer activity probably across the chromatophore membrane [62].

Recently it has been shown that, in etiolated bean leaves greened by brief flashes, the 505 nm carotenoid change is closely correlated with fusion of primary thylakoids; there is a correlation with oxygen evolution but the absorbance change continues when oxygen evolution is inhibited [64].

### 3.3 PHOTOPROTECTION

#### 3.3.1 PHOTOSYNTHETIC TISSUES

The function exhibited by carotenoids in photosynthetic organelles in protecting them against destruction by photodynamic sensitization was first observed in photosynthetic bacteria [65]. A mutant strain of *Rps. sphaeroides*, deficient in coloured carotenoids but accumulating the colourless precursor phytoene (1.36), was destroyed by a combination of light and oxygen but not by either alone, whilst the normal wild-type was not affected by any of these conditions (66). This observation fulfils the criterion of photodynamic sensitization which is 'sensitization of a biological system to light by a substance which serves as a light absorber for photochemical reactions in which molecular oxygen takes part' [67]. In this case the sensitizer is bacteriochlorophyll which itself is usually also destroyed. However, at low temperatures (1°C) it is claimed that wild-type *Rsp. rubrum* and *Rps. sphaeroides* are killed by photodynamic action without significant loss of bacteriochlorophyll [68]. The same photodynamic effect is observed in wild-type *Rps.*

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molecules and with emission of heat into the longer lived ( $10^{-4}$  s) first triplet state  $T_1$  in which the spins of the electrons are no longer paired. Return of the first triplet state to the ground state is accompanied by phosphorescence. (See [184] for review.)

*sphaeroides* in which coloured carotenoid synthesis has been inhibited by diphenylamine [69], and in the photoreaction centre from a carotenoid-less strain of *Rsp. rubrum* [69A].

The lack of protection has been observed in carotenoid-less mutants of *Chlorella vulgaris* [70, 71] and in a pale green mutant of *Chlamydomonas reinhardtii* [72]. Further support for carotenoid participation in photoprotection of algae comes from observing the effect of the herbicides which inhibit carotenogenesis in higher plants [73] and, in particular, the action of SAN 9789 on dividing *Euglena*. This compound specifically inhibits carotenogenesis (see Chapter 4) so that cells of cultures dividing in the light eventually contain so little carotenoid that photoprotection disappears and the cultures die [74].

A large number of carotenoid-deficient mutants of higher plants which are photosensitive have been reported, particularly those from maize [75–77] and *Helianthus annuus* [78, 79] (for further details see Chapter 4).

If germinated seedlings of an albescent mutant of maize are placed in low intensity light (5.382 lx) for five hours, and then illuminated they green up normally; in the absence of the pre-treatment they are bleached. Clearly the low intensity illumination allows sufficient synthesis of carotenoid to stabilize the plastids when chlorophyll begins to be formed [80, 81].

### 3.3.2 NON-PHOTOSYNTHETIC TISSUES

There is evidence that carotenoids in some non-photosynthetic bacteria play a similar role in protecting membranes against photodynamic killing. Such protection was first demonstrated in *Corynebacterium poinsettiae* in the presence of an exogenous photosensitizing pigment, toluidine blue [82]. Later the protective effect was demonstrated without the addition of sensitizers by examining coloured wild-type and colourless mutants of *Micrococcus luteus* (*Sarcina lutea*) [83], a *Halobacterium* sp. [84], a *Mycobacterium* sp. [85, 86], *Micrococcus roseus* [86A] and *Bdellovibrio bacteriovorus* [86B]. The effect appears to be quantitative, for mutants of *M. luteus* with smaller amounts of the same pigments as the wild type are more photosensitive [87]. Wild type *Myxococcus xanthus* grown in the dark produces no carotenoids and on simultaneous exposure to oxygen and light it is killed; however, a mutant which synthesizes carotenoids in the dark is stable to light and oxygen [88]. The action spectrum for the effect is very similar to the absorption spectrum of protoporphyrin IX which is present in the wild-type in a concentration 16 times greater than that in the mutant [89, 90]. No other results on the nature of the endogenous sensitizer have been reported. The protective effect can be demonstrated in isolated membranes of *Acholeplasma laidlawii* [91] and *M. luteus* [92] in the presence of toluidine blue. However, in *Micrococcus roseus* experiments with native and colourless mutant strains and with strains rendered colourless with diphenylamine, the photodynamic



killing was not related to carotenoid content [93]. In fungi, experiments have been reported on *Dacryopinax spathularia* and *Rhodotorula glutinis*. In the former, cells grown in the dark are colourless and on exposure to light and oxygen 89% are killed; in light-grown controls no killing was observed even after four hours illumination [94]. However, photosensitization of *R. glutinis* in the presence of an endogenous sensitizer was not prevented by carotenoids [95, 96]. On the other hand, protection against endogenous sensitization is well marked in the non-photosynthetic dinoflagellate *Cryptocodinium cohnii* compared with a pigment-deficient mutant [97]. Later experiments with *Neurospora crassa* showed that albino mutants and normal strains made colourless by growing them in the presence of  $\beta$ -ionone, were much more sensitive to u.v. radiation than the wild type growing normally [185].

### 3.3.3. MECHANISM OF LETHAL OXIDATION

The nature of the lethal oxidation in photosynthetic bacteria and algae is not known but experiments with the *albina* mutant of *H. annuus* indicated a possible site of action in higher plants. When this mutant is grown in dim light (20 lx) its chloroplasts have a lamellar system with well developed thylakoids (Fig. 3.4). If cells with such chloroplasts are bleached by exposure to bright light (500 lx) then the lamellar system is completely disrupted. A characteristic of this disruption is the loss of chloroplast (70S) ribosomes (Fig. 3.4), the site of chloroplast protein synthesis [98]. The same changes are generally observed in electron micrographs of illuminated leaves of normal plants in which carotenogenesis has been inhibited by various herbicides [99–105]). These experiments have led to the view that the destruction of the protein-synthesizing system is a major site of action of photodynamic killing. Support for this view was obtained from grafting experiments with the *albina* mutant of *Helianthus*. If seedlings of this mutant are grafted on to normal stock, leaf development is blocked at a juvenile stage, a phenomenon which can be observed in normal plants treated with 2-thiouracil, an inhibitor of protein synthesis. However, the grafts grow normally if light of wavelength lower than 450 nm is filtered out of the system [107].

In non-photosynthetic bacteria evidence is accumulating which suggests that the locus of action of carotenoids in photoprotection is the cell membrane but the primary effect is not yet clear. An observed change in permeability in *Micrococcus luteus* (*S. lutea*) is apparently not the primary effect because it is also observed with the antibiotic polymixin and at equivalent levels of killing the antibiotic has a much greater effect on permeability than has photodynamic action [83]. However, a pigmented *Staphylococcus* responds to temperature changes by changing the permeability of its membrane whilst non-pigmented strains do not [108]. On the other hand when the effect of varying the carotenoid content of *Acholeplasma laidlawii* on the membrane lipid fluidity was considered the

Fig. 3.4 (a). Electron micrographs of chloroplasts from seedlings of the *albina* mutant of *Helianthus annuus*. Chloroplast in a leaf cell of a pale green seedling grown at 20 lx; it has a lamellar system with a well-developed granum and ribosomes are numerous.

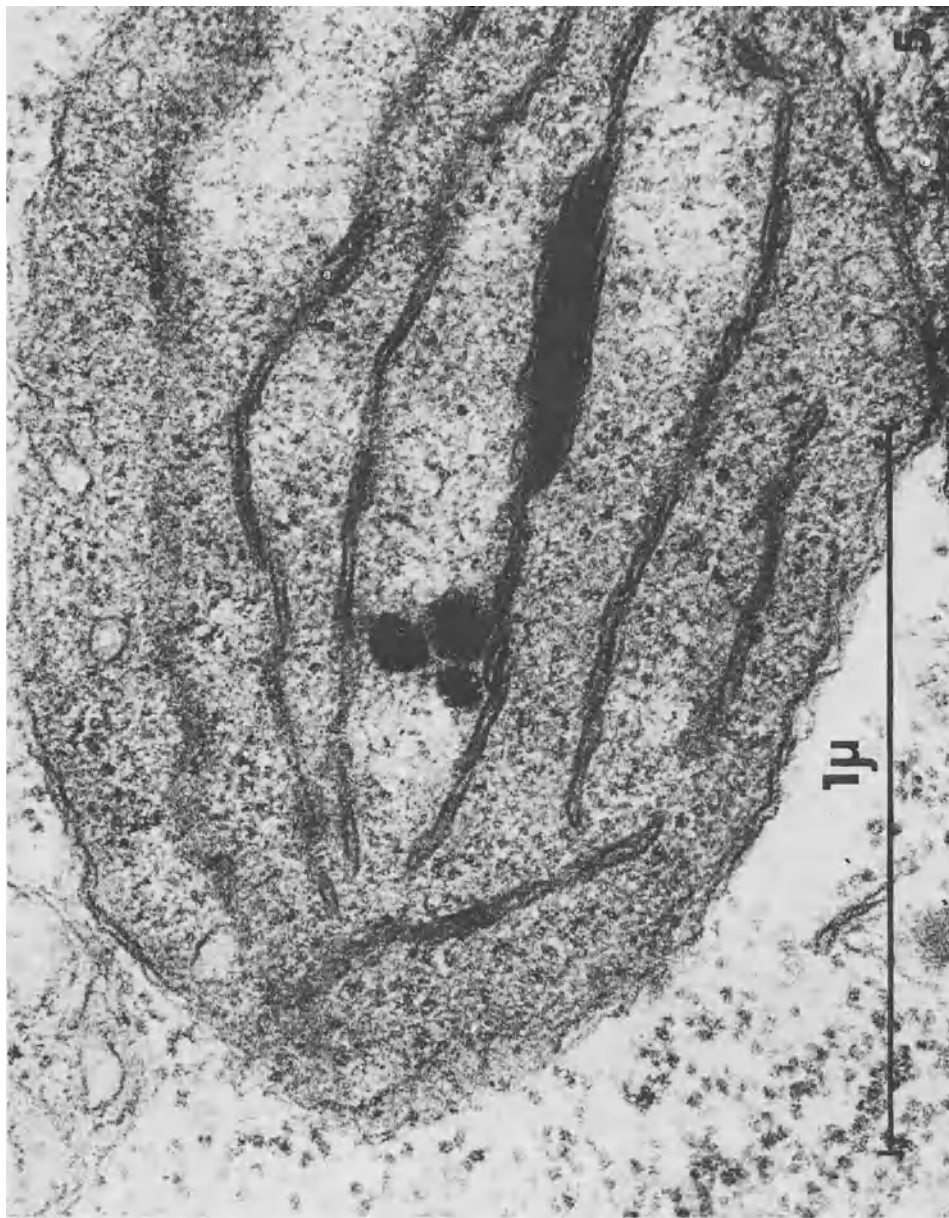
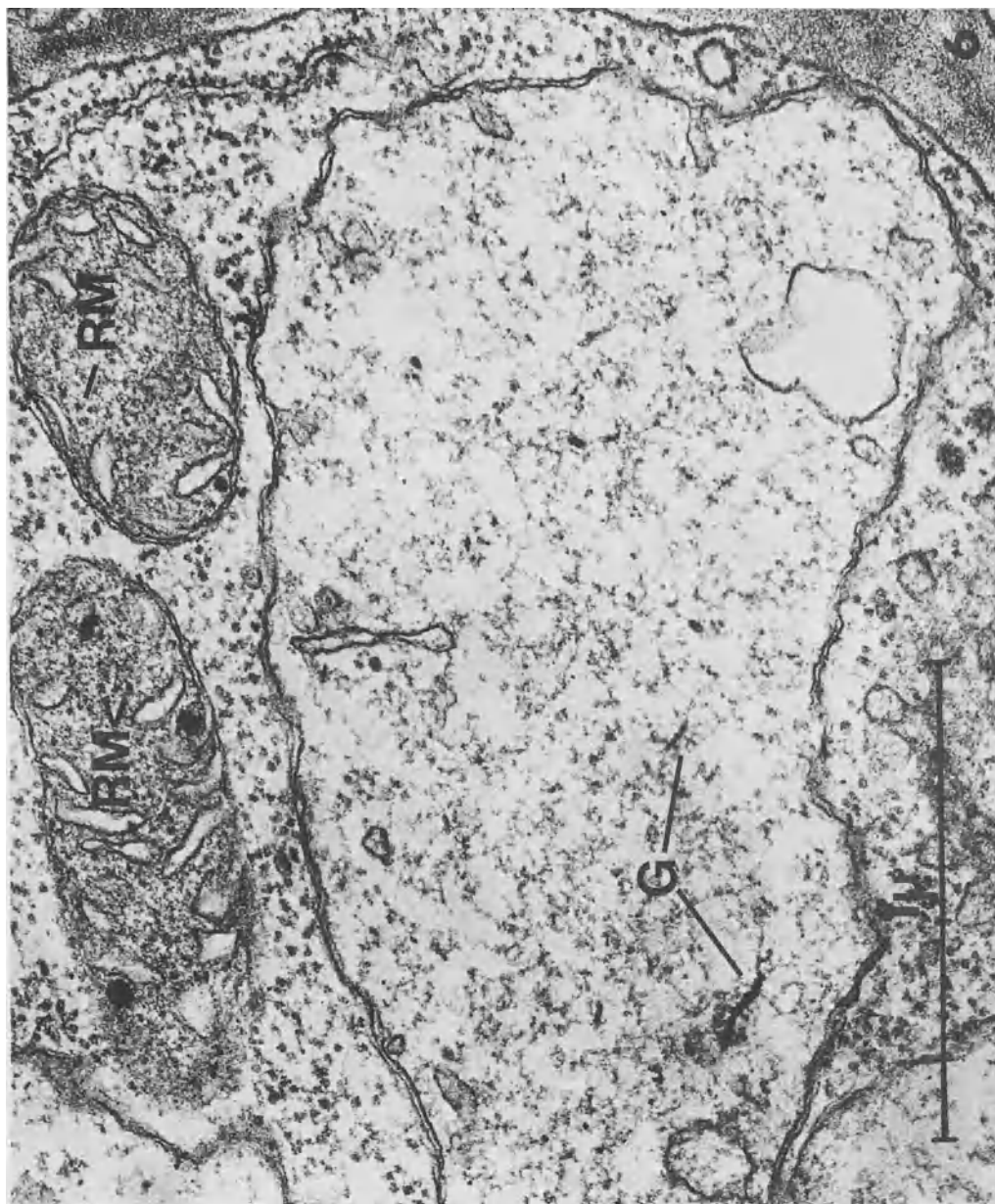


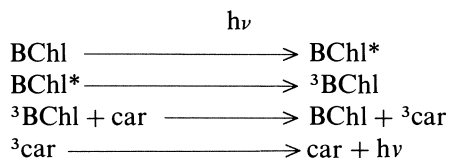
Fig. 3.4 (b). Bleached plastid in a leaf cell of a seedling grown at 500 lx. The lamellar system is completely disrupted and replaced by a few vesicles. The stroma lacks ribosomes and contains strongly electron-dense aggregates [93]. G = DNA fibrils. [Reproduced with permission of Dr B. Walles, who kindly provided original photographs].



conclusion was reached that carotenoids played a part in controlling the rigidity of the membrane [109]; changes in the succinic and NADH oxidases are also affected. The respiratory system geared to the oxidation of malate in the isolated membrane of *M. luteus* is also photodynamically sensitive [87]. The protection appears to be at the menaquinone site [110, 111] and carotenoids associated with phospholipid but not protein are the active components [112]. The significance of the fact that *Mycobact. carotenum* is more tolerant to  $\text{H}_2\text{O}_2$  than are colourless mutants remains to be assessed [113].

### 3.3.4 MECHANISM OF PHOTOPROTECTION

The photodynamic effect in *Rsp. rubrum* involves singlet oxygen and probably superoxide [106]. Singlet oxygen is generated by triplet-triplet energy transfer from reaction-centre bacteriochlorophyll in its triplet state. Carotenoids could then protect by quenching the  $^3\text{BChl}$  state faster than could oxygen. This has now been demonstrated by experiments with reaction centres from a native carotenoid-containing strain of *Rps. sphaeroides* and a carotenoid-less mutant derived from it [46]. In the reaction centres from the mutant,  $^3\text{BChl}$  decays at room temperature with a half-life of about  $6\ \mu\text{s}$  whilst in the carotenoid-containing reaction centre the formation of  $^3\text{car}$  by energy transfer from  $^3\text{BChl}$  takes only 20–30 ns.  $^3\text{Car}$  itself decays with a half-life of 2–6  $\mu\text{s}$ , the energy being dissipated harmlessly to its surroundings. Thus carotene quenches  $^3\text{BChl}$  some  $10^3$  times faster than does oxygen. The overall reaction is



There are also situations in which  $^3\text{BChl}$  cannot be involved although carotenoids are still protective [106]. In these cases singlet oxygen is probably produced and probably quenched directly by carotenoids. *In vitro* evidence supports this view, thus low concentrations of  $\beta$ -carotene (1.13) almost completely inhibit the methylene blue-sensitized oxidation of 2-methylpent-2-ene [114], a reaction which involves singlet oxygen. It is suggested that triplet carotene is formed as the singlet oxygen returns to its ground state, thus:



The  $^3\text{car}$  then dissipates its energy to its surroundings and car is thus available to continue the reaction in cyclic fashion. For this reaction to occur the

transition energy from the ground state to the first excited triplet state of carotene must be near to or below that of the  $^1\text{O}_2 \longrightarrow ^3\text{O}_2$  transition. The calculated value for the triplet state of carotene is  $73.2 \text{ kJ mol}^{-1}$  [114] and the measured value is  $96.2 \text{ kJ mol}^{-1}$  [115]; that for the  $^1\text{O}_2$  level is  $94.1 \text{ kJ mol}^{-1}$  [116]. Calculations show that polyenes with fewer than nine conjugated double bonds would be inefficient quenchers [107] and this has been borne out experimentally with studies with carotenoid mutants of *Chlorella vulgaris* [117–119], *Rps. sphaeroides* [120] and *M. luteus* [87, 121]. The fact that the  $Q_{10}$  over normal temperature ranges is 1.0 [107, 122, 123] is strong evidence that the primary act is photochemical.

A phenomenon which may be widespread and has yet to be explained is the lack of photodynamic protection at low temperatures. Leaves held at  $1^\circ\text{C}$  and illuminated lose their carotenoids and chlorophylls by what appears to be a photo-oxidation mediated by singlet oxygen arising from the quenching of the excited triplet state of chlorophyll by atmospheric oxygen [124]. At the same low temperature wild type *Rsp. rubrum* and *Rps. sphaeroides* are also killed by photodynamic action but in these cases there is no significant loss of chlorophyll [124]. Similarly, the protection by carotenoids is much reduced at  $4^\circ\text{C}$  in *M. luteus* both in the presence and absence of an endogenous photosensitizer [125].

### 3.3.5 PROTECTION AGAINST ULTRA-VIOLET RADIATION

There are two reports which suggest that carotenoids protect against ultra-violet (u.v.) radiation damage but detailed investigations are still required.

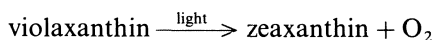
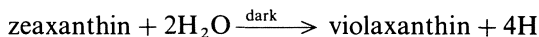
A u.v. resistant strain of the blue-green alga *Gloeocapsa alpicola* contains higher carotenoid levels than does the wild type. If both strains are exposed to u.v. light then the pigment content of the wild-type is reduced whilst that of the resistant cells is unaltered. Similarly, treatments which reduce the carotenoid content of the strains reduce their survival rates on u.v. illumination [126]. Conidia (coloured) from wild-type *Neurospora crassa* are less sensitive to 'black light' (300–425 nm) in the absence of an exogenous photosensitizer than are conidia from *albina* strains. A rather similar reaction is observed to short wavelength u.v. radiation [127].

## 3.4 THE XANTHOPHYLL CYCLE

5,6-Epoxides of xanthophylls are found in all aerobic photosynthetic tissues except the cryptophyte algae and those containing phycocyanins, i.e. blue-green and red algae\*. They are also absent from photosynthetic bacteria. Sapozhnikov [see 128] was first to show changes in epoxide levels in leaves; these levels decreased on illumination in the absence of oxygen and recovered

\* There is one isolated report of epoxides in red algae, see Chapter 7.

in the dark in the presence of oxygen. This has now been confirmed many times and has been studied in detail. It has been suggested that the basic reaction in higher plants, the reversible epoxidation of zeaxanthin (1.18) via antheraxanthin (5,6-epoxyzeaxanthin) to violaxanthin (5,6,5',6'-diepoxyzeaxanthin), is involved in oxygen evolution in photosynthesis [129, 130] thus:



It seems too simple an approach to suggest that this reaction is the source of photosynthetic oxygen in higher plants and algae and in any case, the reaction is probably too slow by at least two orders of magnitude [130] and is not universal in aerobic photosynthesis. It has been claimed, however, that it functions only at high light intensities when  $\text{CO}_2$  fixation becomes limiting [131]. There is a threshold of light intensity below which no de-epoxidation occurs; this varies according to whether plants normally grow in shade or not; for example, the threshold of *Hydrangea* is 800–1000 lx whilst that for maize is 8000–10 000 lx. The light threshold is abolished by salicylaldoxime, which inhibits zeaxanthin epoxidation. Thus it is concluded that both forward and back reactions are proceeding continuously but a net de-epoxidation occurs when the light-stimulated reaction is proceeding faster than the epoxidation reaction which does not appear to be directly affected by light [128, 132].

Studies at the enzyme level are gradually clarifying the details of the situation. The de-epoxidase, mol.wt. 60 000, has a pH optimum of 5.2 and is inactive at pH 7.0 [133]. It is a lipoprotein containing monogalactosyl diglyceride and a functional disulphide group. It is active only on all-*trans*-carotenoids with the 3*S*,5*R*,6*S* configuration [186]. The reductant for the reaction is ascorbic acid and the dehydroascorbic acid so formed is reduced back to ascorbic acid by coupling with a NADPH-dependent glutathione reductase and a glutathione-dependent dehydroascorbic acid reductase [134, 135]. The low pH maximum is also observed *in vivo* [136, 137]. The stimulation of de-epoxidase activity on illumination of chloroplasts is thus due to the drop in pH to around 5.5 caused by the formation of a proton gradient across the thylakoid membrane and which is associated with ATP production [135, 138]. These observations also explain the older reports that *in vivo* the de-epoxidase was stimulated by the addition of ATP or by lowering the pH of the bathing medium and inhibited by compounds, such as the herbicide CCCP, which destroy the proton gradient [134, 136, 138]. There is, however, a claim that de-epoxidation in *Hydrangea hortensis* is inhibited by *o*-diphenol at concentrations which do not inhibit respiration [139]. The action spectrum for the light stimulation just referred to is very similar to the absorption spectrum of chlorophyll *a* [128, 140] and the stimulation is not observed in illuminated

etiolated seedlings until some chlorophyll has been formed [128]. When violaxanthin binds to the enzyme there is a marked change in its absorption spectrum before the molecules undergo any chemical change [145]. The low optimum pH indicates that the enzyme is located on the *inner* surface of the thylakoid membrane [137] and of the stroma lamellae, where it is also found [145].

Zeaxanthin epoxidase which has recently been obtained from lettuce chloroplasts [138] is a mixed function oxidase, that is, it requires both NADPH and  $O_2$  as cofactors [135, 140]. In contrast to the de-epoxidase it has a pH optimum of 7.3 and shows no activity at pH 5.5. *In vivo*, it is not inhibited by illumination and indeed can be stimulated by it [136–141] because of the photoproduction of NADPH. These observations indicate that it is located on the *outer* surface of the thylakoid.

The circumstantial evidence for the apparent physical separation of the two enzymes has led to an attractive proposal for the mechanism involved in the Xanthophyll Cycle (Fig. 3.5) [135]. Because of the different pH optima of the enzymes, the cycle depends on the light-induced proton gradient across the thylakoid membrane and on the movement of zeaxanthin and violaxanthin across the membrane. There is as yet no direct proof of such a movement but the fact that the availability of violaxanthin for de-epoxidation varies with experimental conditions may point to the control of movement of the pigment across the membrane, or possibly to some conformational change which controls the exposure of the lipophilic pigment to the enzyme [142, 143]. Similarly the changes reported in the outer envelope of the chloroplast [187] where violaxanthin accumulates [188] are probably due to exchange between the envelope and the thylakoids and not to enzymic activity in the envelope itself [189].

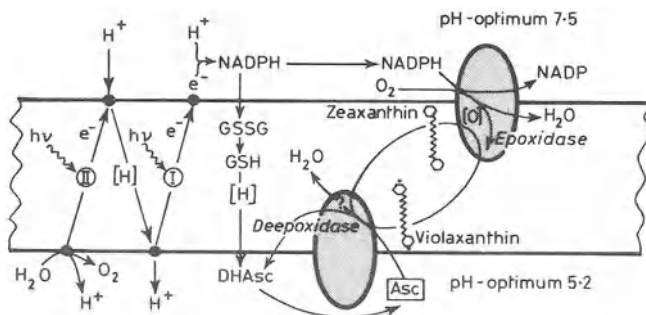


Fig. 3.5. A proposed mechanism for the xanthophyll cycle in chloroplasts (Redrawn from [128]).

The same cycle has been observed in green tomato fruit [144], in ferns and Bryophytes [142] and in certain green algae (Chlorophyceae) [136, 145–147], brown algae (Phaeophyceae) [135] and in *Ochromonas* sp. (Chryso-

phyceae) [135]. A mutant of *Chlorella vulgaris* which does not normally synthesize epoxides does not exhibit the cycle [146], neither is it observed in blue-green algae, red algae or *Cryptomonas* sp. [147], which also do not normally synthesize epoxides [135]. In *Euglena* spp., diatoms (Bacillariophyceae) and in another chrysophyte *Isochrysis*, a xanthophyll cycle is observed [147] but in these cases the acetylenic carotenoids diatoxanthin (2.25) and diadinoxanthin (5,6-epoxydiatoxanthin) are involved. This means that only one epoxidation step occurs: the 5,6-double bond at the acetylenic end of the molecule appears not to be susceptible to the epoxidase enzyme.

As indicated at the beginning of this section the Xanthophyll Cycle is probably not involved in photosynthesis and we are left with the question of the function, if any, of this cycle. The most plausible view at this moment is that it allows the utilization of reducing equivalents at the site of their formation within the chloroplast, which improves the final ATP/NADP ratio [135].

### 3.5 PHOTOTROPISM

Phototropic responses can be observed in all higher plants and in a number of fungi (e.g. Phycomycetes) and the action spectra for the response in various plants suggest superficially that carotenoids are involved. The first action spectrum for the phototropic bending of oat (*Avena*) seedlings was first measured in 1909 [148] but it was not until the 1930s that the view was taken that carotenoids were the mediators in the response [149, 150] and indeed carotenoids, particularly violaxanthin, are present in *Avena* coleoptiles [151]. Similar reports have been published for *Phycomyces* [149]. Later investigations have been centred to a great extent around the rival claims of carotenoids and riboflavin as mediator because the latter also occurs in photosensitive zones and has an absorption spectrum sufficiently similar to that of carotenoids to make it difficult to distinguish between them from the point of view of action spectra, the measurement of which is so much less accurate than is that of absorption spectra. The early difficulties of measuring absorption spectra *in vivo* have now largely been overcome. The experiments of the 1950s and 60s have been critically reviewed a number of times [151–156] and the conclusion must be that at the moment studies based on action spectra are not likely to resolve the carotenoid/riboflavin controversy [157]. Furthermore, later work has revealed that a photoreceptor need only be present in very low concentrations [158] and a calculation from results with 'carotenoid-less' mutants of *P. blakesleeana* indicates that the sporangio-phores contained 0.1  $\mu\text{g/g}$   $\beta$ -carotene and that this was ten times more than necessary for a phototropic response [159]. This meant that  $\beta$ -carotene could not be ruled out as the mediator and that conclusions from previous experiments on 'carotenoid-less' mutants and on organisms in which the carotenoid level was very considerably reduced were likely to be invalid.



Recently, however a mutant containing no detectable  $\beta$ -carotene (less than  $4 \times 10^{-5}$  times the normal level) has been shown to exhibit normal phototropism [160]; this adds considerable weight to the view that  $\beta$ -carotene is not involved in phototropism.

Three other aspects should be briefly mentioned. Firstly, recently measured action spectra show a peak at 370 nm which may be associated with riboflavin but which cannot be associated with carotenoids [161, 162]. Secondly the isolation of a photoactive pigment complex from *Phycomyces* suggests that two pigments might be involved [163]. Thirdly, in *Pilobus kleinii* an *in vivo* absorption band is observed at 500 nm, a wavelength which elicits little photoresponse [164].

### 3.6 PHOTOTAXIS

The problem of phototaxis in algae is still unresolved although the phenomenon was first observed over 90 years ago when it was believed that the photosensitive area was the eye-spot or stigma [165]. It was later considered that in *Euglena* the photosensitive zone was not the eye-spot but the paraflagellar body at the base of the major flagellum [166]. It is now generally accepted that the latter is the photoreceptor, for many organisms without a stigma show the response [165, 167], as does a mutant of *Euglena* which lacks an eye-spot [168], although in this case it is claimed that it also lacks a paraflagellar body [169]. However, in other algae cells which lack stigmata are often less sensitive than those which possess them, whilst cells lacking both stigmata and paraflagellar bodies exhibit no phototactic response [170]. The view first proposed in 1927 that the pigments in the stigma screen the primary photoreceptor and that organisms respond to light in such a way as to minimize such screening [171] appears to be applicable in the case of *Euglena* [172], where the action spectrum [172, 173] does not suggest a carotenoid as a primary receptor. The action spectra for a number of *Pyrrophyta* [174, 175] and for the water mould *Allomyces* [176] suggest that a similar mechanism, not involving carotenoids, is functioning.

Phototaxis in photosynthetic bacteria would appear to be a simpler phenomenon than that in algae and fungi. As long ago as 1888 phototaxis was shown to be related to a change in rate of photosynthesis [165]. The action spectrum for phototaxis was very similar to that for photosynthesis and thus both carotenoids and bacteriochlorophylls are involved [165, 177–179]. Discrepancies in the carotenoid region of the action spectrum for *Rhodospirillum rubrum*, which did not exactly match the absorption spectrum of the major carotenoid component spirilloxanthin (*I.19*), were resolved when it was shown that in young cells used by the photobiologists, spirilloxanthin was by no means the major pigment present although it was in mature cells [180].

## 3.7 SPORANGIOPHORE FORMATION

The formation of sporangiophores in mature *Phycomyces blakesleeanus* mycelia is inhibited in a closed illuminated system in which oxygen is limiting [181]. Blue light reverses the inhibition but in carotene-deficient mutants (car A, car B and car R deficient) the threshold for the blue light effect is raised by 100–2000 times depending on the amount of  $\beta$ -carotene in the mycelium [182]. The raised threshold is overcome by the addition of retinol to the medium and the role of  $\beta$ -carotene in the system may be to provide a source of retinol in the same way as it does in animals [183].

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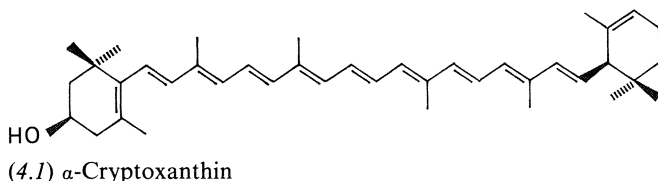
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# CAROTENOIDS IN SEED-BEARING PLANTS—PHOTOSYNTHETIC TISSUES

## 4.1 QUALITATIVE DISTRIBUTION

Various extensive surveys, especially those carried out by Strain [1, 2], have revealed that the leaves of higher plants usually contain the same carotenoids:  $\beta$ -carotene (1.13), lutein (1.74), violaxanthin (1.58) and neoxanthin (1.47). They are occasionally accompanied by smaller amounts of  $\alpha$ -carotene (1.12), mutatochrome [ $\beta$ -carotene-5,8-epoxide (1.44) [3],  $\beta$ -cryptoxanthin (1.60), lutein 5,6-epoxide [4] [ $\equiv$ isolutein [5], eloxanthin [6], taraxanthin [7]] (1.48), zeaxanthin (1.19) and antheraxanthin (1.24). For example,  $\alpha$ -carotene was detected in only 68% of the species examined in an early survey [8]. Even more rarely have other xanthophylls been observed, such as  $\alpha$ -cryptoxanthin [ $\equiv$ zeinoxanthin (4.1)] [9] in lucerne (*Medicago sativa*) [10]. Xanthophylls normally occur unesterified in leaves [11] but during senescence when the chloroplasts are disintegrating the xanthophylls released into the cytoplasm are esterified [12] (see Section 4.10.2).

The colourless polyenes, phytoene (1.36) and phytofluene (1.37) are rather widely distributed [13] but only at levels about one two-hundredth that of  $\beta$ -carotene [13, 14]. In early investigations *cis*-isomers of  $\beta$ -carotene were reported [15], but present day opinion is that they are rarely, if ever, naturally occurring but are more likely to be artefacts of isolation (see Chapter 1).



The constancy of the qualitative distribution in leaves of higher plants is remarkable and applies to the most diverse plants from varying habitats. Strain, who has probably examined more different species than any other

investigator, found no significant qualitative variation between the major leaf carotenoids in, for example, an insect catcher (*Drosera* sp.), a mistletoe (*Viscum angulatum*, Loranthaceae) which parasitizes another mistletoe, a marine plant (*Phylaspadix*, Najadaceae), a salt mangrove, (*Halophila*, Hydrocharitaceae), members of the Compositae (e.g. *Cotula coronobifolia*) and members of the Chenopodiaceae (e.g. *Salicornia ambigua*). He also found that the pattern was maintained when plants endemic to Europe, Asia, Africa and South America were grown in Hawaii or California [1, 2].

This tightly controlled pattern indicates that all the higher plants have evolved from a common ancestor. The importance of carotenoids in leaf metabolism is emphasized by the fact that any mutation which significantly changes this pattern is lethal (see Chapter 3).

## 4.2 QUANTITATIVE DISTRIBUTION

### 4.2.1 INTERSPECIES DIFFERENCES

In spite of the stability of distribution of leaf carotenoids amongst plants there are considerable quantitative variations. The  $\alpha$ -carotene levels can vary from 0 (e.g. nettle, barley) [8] to 35% of the total carotene present (Table 4.1) [16]. The  $\beta$ -carotene level varies between 200 and 700  $\mu\text{g/g}$  dry weight leaf according to species [15]. The xanthophyll distribution in lucerne, a typical forage crop, and cabbage is given in Table 4.2. In contrast to this distribution *Syngonium* (Araceae) contains much neoxanthin (1.47) and little violaxanthin (1.58), *Solidago* (Compositae) much violaxanthin [3] and *Fremontia* (Sterculiaceae) at least as much zeaxanthin (1.18) as lutein (1.73) [1]. In mountain ash grown north of the Arctic Circle only the leaves of introduced plants produce lutein 5,6-epoxide [18]. In a number of dioecious plants examined, the leaves of female plants synthesized more carotenoids than did leaves of the male plants [19].

### 4.2.2 INTRAPLANT VARIATION

Distribution of carotenoids in a plant has generally been confined to measurements on  $\beta$ -carotene, because of its pro-vitamin A activity. Leaves contain some 85–95% of total  $\beta$ -carotene in typical forage plants [20–22]. The pigment is generally distributed fairly evenly through a leaf, although the concentration in the tip is slightly higher than in the rest of the leaf [23, 24], but in pine needles the highest level is in the middle [25]. However, there is considerable difference between the concentration on the oldest and the youngest leaves in the same plant in tobacco [26], turnip [27], *Betula verrucosa* [28], maize [21], and apple [29]. In maize the typical values were 800  $\mu\text{g/g}$  and 547  $\mu\text{g/g}$  (dry wt.) for young and old leaves respectively [21]. Similar differences are observed between upper and lower stem regions [20]. The leaf



Table 4.1 Carotene content of leaves of various trees [16]

	Concentration mg/100 g (dry wt.)		$\alpha$ -Carotene as percentage of total carotenes
	$\alpha$ -Carotene	$\beta$ -Carotene	
<i>Abies cephalonica</i>	4.0	6.4	35.4
<i>Acer campestre</i>	6.8	20.3	22.9
<i>Aesculus carnea</i>	9.8	37.5	20.3
<i>Alnus glutinosa</i>	4.4	22.8	14.6
<i>Bougainvillea glabra</i>	18.8	39.2	30.8
<i>Calycanthus occidentalis</i>	12.1	33.9	25.8
<i>Fagus engleriana</i>	9.5	40.0	18.9
<i>Ficus elastica</i>	3.8	12.4	22.4
<i>Fraxinus excelsior</i>	12.5	26.5	30.6
<i>Gleditschia tricanthos</i>	1.7	24.6	6.4
<i>Hedera helix</i>	10.1	33.6	25.4
<i>Juglans nigra</i>	5.0	31.1	14.0
<i>Liriodendron tulipifera</i>	1.9	27.4	6.0
<i>Magnolia acuminata</i>	10.6	21.7	32.4
<i>Metasequoia glyptostroboides</i>	4.7	23.4	22.8
<i>Picea breweriana</i>	5.3	11.2	32.5
<i>Pinus jeffreyi</i>	5.4	11.1	31.8
<i>Platanus occidentalis</i>	2.1	24.4	7.7
<i>Populus alba</i>	1.6	25.0	5.9
<i>Quercus borealis</i>	2.1	30.3	6.6
<i>Rhus typhina</i>	5.3	33.8	13.5
<i>Rosa centifolia</i>	9.6	28.8	21.5
<i>Syringa vulgaris</i>	7.4	40.1	15.2
<i>Taxodium distichum</i>	10.0	15.6	38.4

Table 4.2 Quantitative distribution of major xanthophylls in lucerne [17] and cabbage [31]

Xanthophyll	Percentage of total xanthophylls present	
	Lucerne	Cabbage
$\beta$ -Cryptoxanthin (1.60)	4	—
Lutein (1.74)	40	61
Zeaxanthin (1.18)	2	—
Violaxanthin (1.58)	34	21
Neoxanthin (1.47)	19	18

blade contains 90% more  $\beta$ -carotene than the petiole [21, 30] which in turn seems to have slightly less than the mid-ribs [20].

### 4.3 LOCALIZATION

Leaf carotenoids are specifically located in the grana of the chloroplasts, probably in the form of chromoproteins. It has been calculated that a maize chloroplast contains  $22 \times 10^7$  carotenoid molecules [32]. A crystalline  $\beta$ -carotene chromoprotein reported some years ago [33] is probably an artefact [34] although more recently protein complexes with violaxanthin [35] and  $\beta$ -carotene [36] have been reported. If chloroplast proteins are solubilized with detergent they can be separated centrifugally into two main fractions – light and heavy. These two fractions correspond to photosystem I and photosystem II in photosynthesis (see Chapter 3). There is a differential distribution of carotenoids in the two fractions,  $\beta$ -carotene being enriched relative to xanthophylls in the light fraction and xanthophylls preponderating in the heavy fraction [37–48]. This is illustrated in Table 4.3. This charac-

*Table 4.3* Carotenoid distribution in two sub-chloroplast particles obtained by detergent (Triton X-100) treatment [46] (values are expressed as moles per 100 moles of total chlorophyll)

Pigment	Light particle	Heavy particle	Intact thylakoids
$\beta$ -Carotene (1.13)	16	6	6.3
Lutein (1.74)	6	16	9.6
Violaxanthin (1.58)	1	3	2.8
Neoxanthin (1.47)	1	2	2.5

teristic distribution can also be demonstrated by electrophoresis of the detergent-solubilized proteins [49]. Sequential extraction of chloroplasts with changing solvents also demonstrates differential removal of carotenoids [50]. The photosystem I particles contain about 13 carotenoid molecules, mainly  $\beta$ -carotene, per hundred molecules of chlorophyll [50A]. A high degree of order is apparent amongst the carotenoid molecules with the long axes of the molecules parallel to each other and to the plane of the thylakoid membrane [50B].

Coloured carotenoids are absent from the osmiophilic droplets of chloroplasts [51, 52] in contrast to the situation in chromoplasts (Chapter 5). The bundle sheath agranal chloroplasts of *Sorghum* [53, 54], *Zea* [54–56] and *Pennisetum setaceum* [54] are enriched in carotene compared with the mesophyll chloroplasts. The fact that the reverse situation exists in *Elusine coracana* [54] is possibly due to the presence of grana in the bundle sheath chloroplasts. In spinach chloroplasts, the stroma lamellae contain the same amount of  $\beta$ -carotene as the grana stacks but the latter contain much more

xanthophyll [57]. The chloroplast membrane envelope of spinach apparently contains mainly violaxanthin [58]. Recent biophysical studies suggest that in chloroplast membranes carotenoids are oriented at a flat angle ( $-16^\circ$ ) [59].

A red pigment, presumably carotenoid, is present in droplets in the chloroplasts of the bronze winter leaves of *Thuja ericoides* [60].

#### 4.4 ETIOLATED SEEDLINGS

Seedlings germinated in the dark contain small amounts of plastid xanthophylls and relatively little, if any,  $\beta$ -carotene; in etiolated maize, for example, no  $\beta$ -carotene can be detected [61, 62]. This phenomenon is further emphasized by the observation that the xanthophyll: carotene ratio in green barley leaves is 2.8:1 whereas that in etiolated leaves is 15.5:1 [51]. Further, in the etiolated barley seedlings some 25% of the 'carotenes' is  $\beta$ -carotene-5,6,5',6'-diepoxide [63]. The same pattern is observed in wheat seedlings [64] and mung bean seedlings [65]. In some normal non-etiolated alpine plants the xanthophyll:carotene ratio can also reach 15:1 [66].

In pine seedlings, which produce green cotyledons, the situation is different; although more pigment is produced in the light than in the dark, the xanthophyll:carotene ratio is of the same order in both cases, which is a reflection of the synthesis of chloroplasts in the dark [67]. Even so there is an indication in some species of pine that relatively less carotene is formed in the dark (Table 4.4). Both  $\alpha$ - and  $\beta$ -carotenes are formed but relatively more  $\beta$ -carotene is formed in the dark than in the light (Table 4.4).

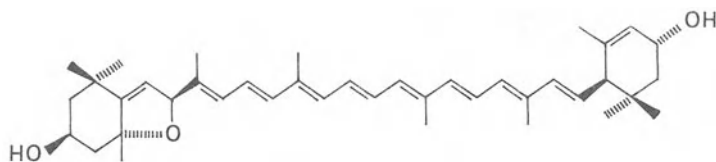
Table 4.4 Relative amounts of carotenoids in pine seedlings grown in darkness and in light [67]

Species		Ratios	
		$\frac{\text{Xanthophylls}}{\text{Carotenes}}$	$\frac{\beta\text{-Carotene}}{\alpha\text{-Carotene}}$
<i>Pinus jeffreyi</i>	Dark	1.96	3.29
	Light	1.79	1.66
<i>P. silvestris</i>	Dark	2.63	4.19
	Light	1.37	3.70
<i>P. radiata</i>	Dark	3.69	5.27
	Light	1.72	2.73
<i>P. contorta</i>	Dark	1.87	5.55
	Light	1.75	2.78

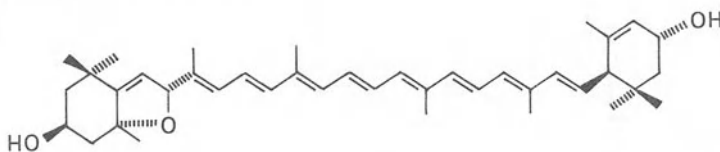
The pigments produced in darkness are considered to be located in the etioplasts and the carotenoid changes which occur when these are converted by light into chloroplasts are discussed in Section 4.7.1.

The main xanthophylls of the cotyledons of etiolated French bean seedlings

are not those found in true leaves, but consist mainly of auroxanthin (1.62) and chrysanthemaxanthin (4.2) (Table 4.5) [68] and similarly, more oxidized pigments appear as mung beans germinate in the dark [65].



(4.2) Chrysanthemaxanthin



(4.3) Flavoxanthin

Table 4.5 Pigments in cotyledons and true leaves of five-day etiolated French bean (*Phaseolus vulgaris*) seedlings [68]

Pigment	% of total carotenoids present	
	Cotyledons	True leaves
$\beta$ -Carotene (1.13)	10.7	10.8
Lutein (1.74)	8.3	38.4
Chrysanthemaxanthin (4.2)	23.6	0
Flavoxanthin (4.3)	46.0	0
Neoxanthin (1.47)	–	50.7
Unknown A	11.4	0
Unknown B	–	trace

## 4.5 MUTANTS

Mutations which involve the deletion of the synthesis of carotenoids in chloroplasts are lethal. A combination of oxygen and light causes the photo-destruction of the chlorophylls in chloroplasts which lack  $\beta$ -carotene. Such mutants will grow in very dim light or in darkness heterotrophically.

### 4.5.1 MAIZE

Maize plants have been studied in detail from the point of view of carotenoid mutants. A maize mutant homozygous for the gene  $cl_1$  produces white or pale yellow seeds which give rise to albino seedlings which contain no  $C_{40}$  polyenes [69]. A dominant modifier nuclear gene  $cl_M$  also exists, certain

alleles of which partly or completely suppress the albinism induced in seedlings by *cl*<sub>1</sub> [69, 70].

An albino mutant (*w*-3) of maize accumulated phytoene (1.36) in its leaves together with smaller amounts of phytofluene (1.37),  $\zeta$ -carotene (1.14) and neurosporene (2.14) [71]. The mutation is in a gene on maize chromosome 2 [72].

Other white-seeded mutants have been examined: *w*-3 60-1201-7+ and *w*-3 59-3024-25 + *n* synthesize phytoene and phytofluene, whilst *w*-3-*ch*/2 60-1047-194+ produces only phytoene [73]. These mutants occasionally produce yellow seeds which, on germination, synthesize the expected polyenes, although under certain conditions they can also synthesize chlorophylls. In all cases, the polyenes were located in the 'chloroplasts'. A pastel mutant (*pas* 8686) accumulates carotenoid precursors and carotenoids themselves in reduced amounts [74]. The phenotype is also temperature sensitive; at 22°C it produces 7.9% as much carotene and 45% as much xanthophyll as normal seedlings; the corresponding figures at 37°C are 61.4 and 144.7% [75]. Another temperature-sensitive mutant of maize has also been described which produces only one third the amount of carotenoid at 15°C than at 27°C, at which temperature it produces normal amounts [76].

Two further mutants of maize have been described; one was said to accumulate lycopene [77-79] and the other  $\zeta$ -carotene [79-81]. Later experiments indicate that lycopene represents only 5% of the total carotenoids whilst  $\zeta$ -carotene represents about 50% (Table 4.6) [82].

An albescent mutant of maize exists in which the seedlings are very low in carotenoids; however, if the germinated seeds are placed in low light intensity (5.382 lx) for five hours, the plastids are stabilized in the sense that on illumination they green up normally. In the absence of this pre-treatment the seedlings are bleached [83, 84].

Table 4.6 Relative amounts of carotenoids in leaves of lycopene and  $\zeta$ -carotene mutants of maize grown under low light intensity [82]

Pigment	Percentage of total carotenoids present	
	Lycopene mutant	$\zeta$ -carotene mutant
Phytoene (1.36)	13.1	31.0
Phytofluene (1.37)	7.8	18.0
$\alpha + \beta$ -Carotene (1.12, 1.13)	2.1	—
$\delta$ -Carotene (4.4)	48.4	—
$\gamma$ -Carotene (1.38)	8.2	—
Lycopene (1.2)	5.4	—
$\zeta$ -Carotene (1.14)	—	51 <sup>a</sup>
Antheraxanthin (1.24)	13.6 <sup>b</sup>	—

<sup>a</sup> Also some *cis*-isomers present.

<sup>b</sup> Other xanthophylls also present.

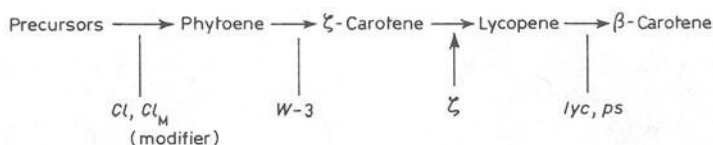
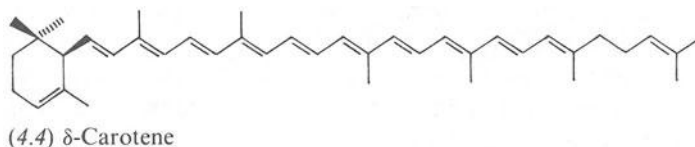


Fig. 4.1. Nuclear control of carotenoid synthesis in maize seedlings.



All these observations suggest the nuclear control of carotenoid synthesis in maize outlined in Fig. 4.1. In the lycopene mutant the amounts of polyenes present are of the same order as those observed in normal leaves whilst the  $\zeta$ -carotene mutant contains twice as much. All these maize mutants are of course photosensitive and it has been suggested that as the pigment-complex has different properties from that of normal leaves the functional pigment-protein complex does not exist in the chloroplast [79, 86]. This may be linked with the fact that the absence of  $\beta$ -carotene appears to be the controlling factor in whether or not the plant is photolabile [87]. The protein to which  $\beta$ -carotene is normally attached may still be synthesized in these mutants but pigments other than  $\beta$ -carotene cannot be attached to it. It is significant that the lycopene mutant grown either in low light intensity for 6–7 days (25–30 lx) and then bleached in high light intensity for 2–4 days (500–300 000 lx) or grown continuously in 500 lx contained chromoplasts rather than chloroplasts, that is, the organelles had fine structure similar to the chromoplasts in tomato fruit [81, 87].

#### 4.5.2 TOMATO

The tomato phenotype ghost (*gh*) accumulates phytoene (1.36) in its leaves [88] and *albina* and *xantha* mutants of *Lycopersicon* have been described which accumulate 2% and 10% respectively, of the normal chloroplast carotenoids [89].

#### 4.5.3 HELIANTHUS

Two photolabile mutants of *Helianthus annuus* have been described [90, 91]; the yellow (*xantha*) mutant contains about one half the usual amount of chloroplast xanthophylls but no  $\beta$ -carotene [92], whilst the white (*albina*) mutant contains no coloured carotenoids [91, 92]. It is not known whether the latter mutant synthesizes phytoene. Both mutants show Mendelian in-

heritance and each is controlled by a single recessive gene [91]. Another sunflower albino mutant accumulates more carotene than usual in its leaves and it is thought that the metabolic block is in the further metabolism of carotenes to xanthophylls [93].

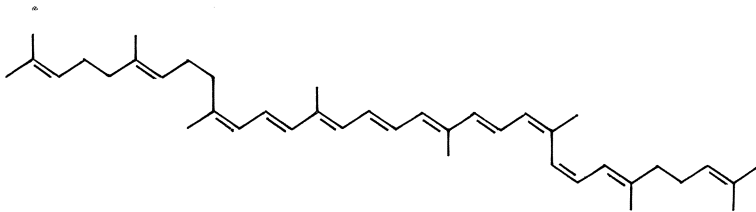
#### 4.5.4 BARLEY

A number of *xantha* and *tigrina* mutants of barley have been examined for carotenoids during germination in the dark (Table 4.7). In all cases the amount of pigment produced was quantitatively similar to that produced by the wild type but in all the mutants except *tig-d*<sup>12</sup> the carotenes represented

Table 4.7 Carotenes and carotene epoxides in etiolated seedlings of barley mutants [63] (pigments expressed as percentages of total carotenoids)

<i>Polyene</i>	<i>Wild-type</i>	<i>Xan-4</i> <sup>21</sup>	<i>Tig-b</i> <sup>23</sup>	<i>tig-n</i> <sup>32</sup>	<i>tig</i> <sup>33</sup>	<i>tig-O</i> <sup>34</sup>
Phytofluene (1.37)	trace	4*	24*	9*	6*	trace
$\beta$ -Carotene (1.13)	79	—	13	14	15	7
$\zeta$ -Carotene (1.14)	—	—	50	13†	22†	—
Proneurosporene (4.5)	—	9	—	5	5	—
Poly <i>cis</i> lycopenes	—	24	—	10	17	—
Lycopene (1.2)	—	4‡	—	50†	35†	71
$\beta$ -Carotene-5,6,5',6'-diepoxide	21	—	7	trace	trace	—
Unknown	—	—	6	—	—	—

\* Probably an overestimate; † plus an unidentified pigment; ‡ neo-(*cis*) lycopene also present.



(4.5) Proneurosporene

a much higher percentage of the total. In *tig-d*<sup>12</sup> the carotenes are qualitatively and quantitatively the same as in the wild type [63]. The significance of these mutants for biosynthetic studies has been considered in Chapter 2.

#### 4.5.5 MISCELLANEOUS

It is claimed that in sugar beet increased ploidy increases carotene levels in leaves [94] and this is true in general of *Lychnis chalcedonia*, *Dianthus barbatus* and *Saponaria officinalis* [95]. In-breeding of cabbage Slava 231 reduces the carotenoid level of the leaves [96].

A chlorophyll mutant of *Antirrhinum majalis* contains the usual chloroplast

carotenoids in lowered amounts [97] and this is also the case, especially with the xanthophyll fraction, in the lutescent mutant of the peanut *Arachis hypogaea* [98]. A temperature-sensitive mutant of lucerne synthesizes much less carotenoid at 10°C than it does at 27°C [99]. Lower levels of carotenoids have been reported in mutants of jute (*Corchorus olitorus*) [100].

## 4.6 SYNTHESIS IN GERMINATING SEEDLINGS

### 4.6.1 EARLY STAGES

Ungerminated seeds generally contain traces of xanthophylls but little or no carotenes [101] although in some cases the amounts of both groups can be considerable, as in maize (Chapter 5). New synthesis generally begins within 3–5 days of germination and continues rapidly during the early period of active growth. For example, a three-fold increase occurs within three days of germination in soya beans [102], and within ten days in wheat seedlings [103]. In a number of pulses and seedlings the amount doubles within seven days [104]. The increase in carotenoids does not always follow the increase in dry matter, so that in some cases, a relative drop can occur in the early stages of germination [see e.g. 105]. A detailed pattern of development has been reported for mung beans (Table 4.8) in which the rapid formation of chloroplast carotenoids can be clearly followed [65].

Table 4.8 Carotenoids of mung bean seedlings grown in the dark for 4 days, then transferred to light for a further 4 days [65]

	4 days dark	1 day light	2 days light	3 days light	4 days light
Phytofluene (1.37)	1.2	4.2	3.9	5.5	13.8
$\alpha$ -Carotene (1.12)	—	1.7	6.0	6.9	17.3
$\beta$ -Carotene (1.13)	2.2	28.2	117.1	278.7	352.1
$\beta$ -Carotene-5,6-epoxide	0.5	1.9	6.2	6.6	15.6
$\beta$ -Carotene 5,6,5',6'-diepoxide	0.5	2.0	14.0	5.3	16.8
Lutein 5,6-epoxide	—	—	—	—	67.3
Violaxanthin (1.58)	—	23.8	130.0	119.6	90.3
Lutein (1.74)	6.8	84.9	321.4	574.4	731.6
Flavoxanthin (4.3)	6.9	—	—	—	—
Auroxanthin (1.62)	2.9	—	—	—	—
Neoxanthin (1.47)	—	11.1	62.9	128.4	184.2
Total carotenoids	21.9	157.8	661.5	1125.4	1489.0

The highest carotenoid levels are always observed in the flag leaf of all wheat genotypes [106]. There is a differential rate of increase; in soya beans, for example, after 54 hours there is three times more carotene in the cotyledon



than in the hypocotyl [102], whilst in the grapefruit (*Citrus paradisi*) germination of the seedlings until the radicle was 1–2 in long resulted in the carotene content (mg/100 g dry wt.) increasing from 3.36 to 44.8 in the embryo and from 0.02 to 0.27 in the whole seedling [107]. It is interesting that two-week-old carrot seedlings will incorporate [ $^{14}\text{C}$ ]-acetate only into squalene, the sterol precursor; only after four weeks does it appear in the carotene precursors, phytoene and phytofluene, and in  $\beta$ -carotene itself [108].

#### 4.6.2 LATER STAGES

Carotenoid synthesis continues rapidly during the early period of active growth. This has been demonstrated many times in many plants in many countries [e.g. USA [26, 27, 103, 109–115], USSR [29, 116–139], Finland [140], UK [141], South Africa [142], Guatemala [143], Canada [144], Australia [145], Germany [23, 146, 147], Japan [148, 149], Norway [150], Poland [151, 152], Egypt [153] and India [154]] that at the time of maximal growth rate the carotenoid level (usually only carotene was measured) was maximal.

The time of maximal concentration is five weeks after drilling with oats and eight days after the plant appears above ground with French beans. Although the carotene concentration begins to drop quite early in the development of a plant, the total amount continues to increase for some time, and it is only at the later stages of maturation that it begins to drop. This is well illustrated for maize in Fig. 4.2.

Legumes also show this drop in older plants but not quite so markedly [155]. The increase in carotene content in soya bean leaves grown in the USA continues beyond maturity until three weeks after the appearance of the first flowers [156]. In Australia, although leguminous crops retain their maximum concentration of carotenoids longer, the level at full maturity is as low as that of any other forage crop [145]. Leaves of late maturing peas contain more carotenoids than those of early maturing strains [157]. It is interesting that in poinsettias (*Euphorbia pulcherrima*)  $\beta$ -carotene is at its highest level during the pre- and intermediate colour stages and decreases during the flower developmental stage of the bracts [158]. In *Vaccinium myrtillus* the  $\beta$ -carotene:lutein ratio changes with the season [159]. In evergreen trees, carotenoid levels in leaves reached their maximum only after several years growth [160]. In species such as spruce two maxima (summer and winter) of carotene level are frequently observed [161] although in some experiments no changes were noted [162], and trees grown in the far north of Russia have one maximum which occurs in the autumn [163].

Evergreens tend to increase their carotenoid levels even when leaf formation is completed. The level drops temporarily during flowering [124].

From the agricultural viewpoint it is important to realize that in pasture grasses which produce a second cutting the carotene values frequently recover to near the original levels [113, 164] but this is not the case with big blue

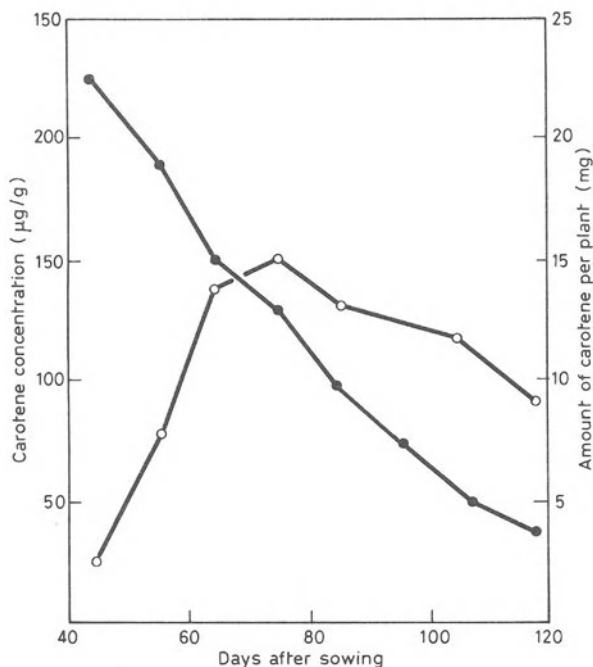


Fig. 4.2. Variation in the carotene concentration (●—●) and carotene content (○—○) in developing maize [21].

stem (*Andropogon furcatus*) or buffalo grass (*Buchloe dactyloides*) [164]. Occasionally late cuttings have higher carotene levels than early cuttings [21] but this is not a universal phenomenon [165]. Frequent clippings can increase the yield per acre [166]. There is often a marked drop in the carotene levels of crops which can be overwintered, such as sprouting broccoli and kale, but this is not observed in Swiss chard [111]. The carotene level in winter wheat also drops at the end of the winter but soon begins to rise [167]. The best time for harvesting clover is at the beginning of bud formation [168] and the maximum level in perennial grasses in the USSR is either at flowering time [169], at tillering [135], or, in yellow trefoil (*Medicago falcata*), at the binding stage [170].

#### 4.7 EFFECT OF ENVIRONMENT ON SYNTHESIS IN LEAVES

##### 4.7.1 LIGHT

###### (a) Etiolated seedlings

Carotenoids, mainly xanthophylls, are present in etioplasts [171] in both the

prothylakoids and the prolamellar body [171A]. On illuminating etiolated seedlings there is a rapid synthesis of chloroplast carotenoids as the seedlings green up. Good examples of this are tobacco [172] and maize seedlings [173]. In the case of *Zea mays* seedlings greening is obvious after 12 hours of exposure to  $50 \text{ J s}^{-1} \text{ m}^{-2}$ , but no greening is observed with five times this light intensity [173]. As there is very little  $\beta$ -carotene in most etiolated seedlings greening results in a drop in the xanthophyll:carotene ratio of about 15:1 to the usual 2–3:1 of normal green tissues [62–65, 173–175]. This does not indicate a preferential stimulation of carotene synthesis as is sometimes suggested [82, 176–178]. A complete pattern of change in illuminated etiolated mung beans is given in Table 4.8 (p. 105) [65]. The stimulation of carotenogenesis on greening is inhibited by antibiotics, such as actidione, which inhibit the development of chloroplasts [179]. The synthesis of carotene is proportional to leaf area in greening etiolated beans [180].

*Phytochrome control.* Carotenoid synthesis is controlled by phytochrome [181–185]. If etiolated maize seedlings are exposed to a flash of red light and returned to darkness, then on illumination the lag period, usually observed before carotenoid synthesis begins, disappears [180]. This effect is abolished if the exposure to red light is followed by a short exposure to far-red light. The abolition of the lag period is probably due to the synthesis in the dark of enzymes concerned with carotenogenesis. There is also an overall stimulation of synthesis which, in the early stages is greater than the

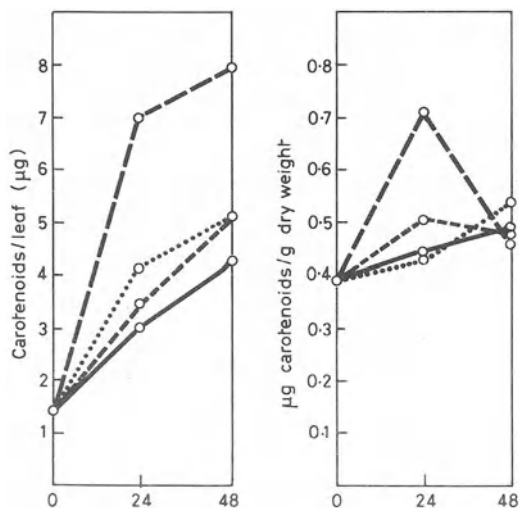


Fig. 4.3. Effect of red and far-red light on carotenoid production by 4-day old etiolated maize seedlings [181]. ○—○, darkness; ○---○, red light (10 min) and then darkness; ●---●, far-red light (10 min) then darkness; ○---○, red light (10 min) followed by far-red (10 min), then darkness.

stimulation of growth (Fig. 4.3). A somewhat similar result is observed with mustard seedlings grown either in the dark or continuously under far-red irradiation [184] which maintains a constant level of the effective Pfr form of phytochrome. After a lag period of three hours the seedlings then begin to synthesize carotenoids at a faster rate than those kept in the dark. Actidione inhibited the effect and this observation emphasizes the view that carotenoid synthesis depends on continuous synthesis of enzymes. Plastid formation and carotenoid production are closely correlated and the phytochrome effect on carotenogenesis can be considered as a response to growth and development of plastids [186–188]. Similar effects of far-red irradiation have been observed with barley (*Hordeum*) and radish (*Raphanus*) seedlings [186–188]. In bean seedlings the phytochrome effect is more pronounced on the xanthophyll than the carotene fraction [189].

Continuous blue light is more effective than red light in bean [190] and barley seedlings [191]. The action spectrum for  $\beta$ -carotene synthesis in illuminated etiolated wheat leaves corresponds to the absorption spectrum of chlorophyll *a*, [189]. Light-induced carotenogenesis in *Raphanus sativus* seedlings is inhibited reversibly by ethanol [192]. Isonicotinic hydrazide (INH) inhibits greening and thus carotenogenesis in wheat and barley [193].

#### (b) Normal seedlings

The effect of light on carotenoid levels in germinating seeds varies with the species examined and some of the effects are summarized in Table 4.9.

Table 4.9 Effect of light intensity on carotenoid production in various plants

Species	Effect	Ref
<i>Secrecea purpurea</i>	Decreases in limited daylight	194
Coffee	Increases in shade compared with full sunlight	195
Spinach	60W/m <sup>2</sup> better than 30W/m <sup>2</sup>	196
Bean	6460 lx: optimum	197
Maize	Change from 1.2 to 32 Jm <sup>-2</sup> : no effect	198
Pea	Change from 1.2 to 32 Jm <sup>-2</sup> : decreases	198
Hydrangea	Complex effect on induction period and levels at steady state	199
<i>Fagus sylvatica</i>	Little difference between sun and shade leaves	200
Apple	Little difference between sun and shade leaves	201

The general assumption that light affects synthesis of all carotenoids equally has been challenged by the observation that high intensities favour zeaxanthin (1.18) and violaxanthin (1.58) synthesis in maize seeds and reduce  $\beta$ -carotene (1.13) and lutein (1.74) formation [202]. The effect on violaxanthin has been confirmed in a number of halophytes [203]. In wheat etioplasts,

however, light stimulated synthesis of  $\beta$ -carotene, lutein, violaxanthin and neoxanthin (1.47) and lowered the levels of antheraxanthin (1.24) and zeaxanthin (1.18) [188], and the indication is that the xanthophyll:carotene ratio is marginally higher in shade than in sun plants [204].

Little is known about the effect of quality of light on carotenogenesis although it is stated that at a constant energy of  $50 \text{ W m}^{-2}$ , red light stimulates synthesis in wheat whilst blue light is most effective in cabbage [205]. There are also claims that midday light, containing a high proportion of short-wave radiation, is more effective than morning or evening light [206, 207].

*Photoperiod.* It was shown some forty years ago that the leaves of plants (soya, cosmos, salvia) growing under a seven hour light photoperiod contained more carotene than those growing under a 14 hour regime [208]. This view was later confirmed using different branches of the same plant, although the age factor which operates from leaf to leaf (Section 4.2.2) was not taken into account [209]. Earlier, an investigation involving a smaller variation in photoperiod (9–14 hours), revealed no effect on carotene synthesis when other factors, such as temperature and moisture, were optimal [210].

*Diurnal variation.* Evidence exists which suggests that a circadian rhythm for carotenoid levels exists in leaves, but the quantitative aspects seem to vary with the plant under consideration. It appears to be generally but not unanimously agreed that a minimum exists at midday [106, 209, 211, 212]. There is one report that in beans the maximum is at midday [213] and one claim that no diurnal variation exists [214]. Where a circadian rhythm is observed the maximum generally occurs in the morning as in rye grown hydroponically [215], spruce [161] and forage plants [216]. In the latter case one pigment, violaxanthin, reverses the general pattern and reaches its peak in the evening. Introduced mountain ash varieties showed a greater carotenoid diurnal variation than local trees [217]. Temperature fluctuation has no great effect on the diurnal variation in fescue [218].

#### 4.7.2 TEMPERATURE

One detailed investigation has been carried out on the effect of temperature on carotenogenesis in relation to the normal photoperiodicity of the tomato plant (Table 4.10). If the night temperature is maintained at  $17^{\circ}\text{C}$  and the day temperature varied then carotenoid synthesis decreases as the temperature is lowered so that a day temperature of  $4^{\circ}\text{C}$  is equivalent to darkness. At constant day temperature ( $17^{\circ}\text{C}$ ) variation in night temperature has little effect on carotenogenesis, although at higher temperatures the leaves are lighter coloured because of marked changes in the anatomy of the leaf [219]. The marked effect of altering the photo-temperature compared with the nycto-temperature is not fully compatible with the earlier observation that the temperature coefficient ( $Q_{10}$ ) for carotenogenesis in isolated bean

Table 4.10 The effect of temperature on carotenoid synthesis by tomato leaves [219]

Temperature (°C)		Pigments, µg/g dry wt.	
Day	Night	Carotenes	Xanthophylls
4	17	121	668
6	17	763	1660
10	17	968	1813
17	17	1331	2492
20	17	1408	2799
23	17	1552	3341
30	17	1633	3550
37	4	1076	2147
17	10	1313	2912
17	23	1315	2444
30	30	1588	3185

leaves is 2.9 in the dark and 1.4 in the light [220]. It is claimed that leaves exposed to low temperature for three hours before extraction of pigments showed raised levels of mono-epoxides and lower levels of di-epoxides, whilst  $\beta$ -carotene levels did not differ from those in controls not subject to cold treatment [221]. In potato spp., frost-resistant plants contained more easily extractable carotenoids in their leaves than did frost-sensitive plants [222].

#### 4.7.3 NITROGEN METABOLISM

A number of early investigations demonstrated a positive relationship between crude protein [113, 141, 223–230], non-protein nitrogen [230] and ammonia-nitrogen [229] and carotenogenesis in forages and seedlings. The results of a typical experiment are illustrated in Fig. 4.4.

Amide-nitrogen and amino acid-nitrogen remained constant during this period [229]. The changes in nitrite levels on dull and sunny days are said to correlate inversely with the carotenoid levels of leaves [231]. An equation has been derived to calculate the carotene content of a forage from the measurement of its total nitrogen content [232].

#### 4.7.4 SOIL NUTRIENTS

##### (a) General

The conclusion of early workers that conditions which lead to the development of a healthy plant also favour maximal carotene production [233, 234] has not been seriously challenged during the past 25–30 years. Plants cultivated under normal fertilizer conditions are not likely to increase their carotenoid content by addition of extra fertilizers [233, 235–242] but some

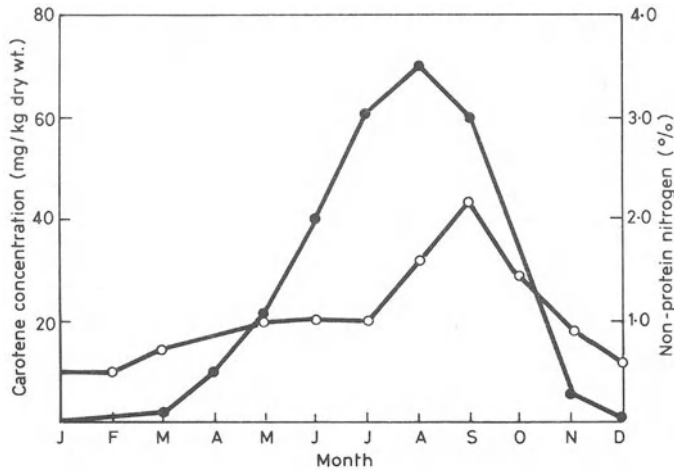


Fig. 4.4. Correlation between carotene concentration (●—●) and non-protein nitrogen concentration in the developing carrot [230].

reports do indicate an improvement under these conditions [243–248]. Sub-normal availability of nutrients obviously affects carotenoid production [116, 245, 249–253], but in general, it is clear that variations arise far more from environmental and climatic factors than from fertilization differences [116, 236, 254–256]. Spraying of fertilizer on plants effectively increases carotenoids in maize leaves [257]. It is said that vacuum infiltration of fumaric or malic acid (3 mM) into 8 day old barley seedlings increases the carotenoid levels [258]. As the same effect is observed with chlorophylls the action is probably on chloroplast formation.

#### (b) Nitrogen

Under artificial conditions some contradictory results have been reported; thus in many cases in sand cultures nitrate is a better source of nitrogen than is ammonium [259], but the reverse is true in tobacco leaves [260]. However, with ammonium, the accompanying anion may have a marked additional effect [261]. Carotene production in cress germinated on ashless filterpaper is decreased by the addition of ammonium chloride, sulphate and phosphate; on the other hand, ammonium bicarbonate, acetate and succinate had no such effect. The depressing effect of the sulphate is reduced by the presence of sodium succinate, malate or aspartate but not by the free acids, which themselves have no effect on carotenogenesis. When sodium nitrite was fed to tobacco plants there was an increase in total carotenoid production but the xanthophyll:carotene ratio fell, which indicated preferential synthesis of carotenes [23]. Grasses respond to nitrogen [262–264], the best values for orchard, Kentucky blue and meadow fescue being obtained with

100 kg/ha [262, 263], as do the leaves of lemon trees [265] and tomato plants [266]. In the case of spinach, celery, cauliflower and cabbage the optimal level of nitrogen was 80 kg/ha [267]. Urea increases carotenoid levels in rye grass (*Lolium perenne*) [268] and pine buds [269]. In the former, carotenoid production is proportional to the urea added [268]. The addition of the nitrogen-fixing *Azotobacter* to maize seedlings cultured in nutrient solution increased the carotenoid content of the seedlings [270].

#### (c) *Phosphorus*

Variations in soil phosphorus levels have no direct effect on carotenogenesis [106, 251, 252, 271, 272] and attempts to correlate carotene levels with different soil phosphorus levels have not been particularly fruitful [273, 274]. However, low phosphorus levels increase the relative amount of zeaxanthin (1.18) and lower that of violaxanthin (1.58) in sunflower leaves and, although no effect on  $^{14}\text{CO}_2$  incorporation into carotenoids was noted [275–277], total carotenoid levels were reduced [278]. Superphosphate slightly reduced carotenoid levels in cabbage [279].

#### (d) *Potassium*

Almost every possible conclusion has been reached on the effect of potassium status on carotenoid formation: a deficiency reduces [109, 280] or increases [281], a moderate addition improves [265, 270, 271, 282], an excess inhibits [233, 245, 253, 264, 283] and no controlling effect is exerted [249, 251, 252]. Later work suggests that potassium deficiency decreases overall carotenoid formation in sunflower leaves but increases  $\beta$ -carotene (1.13) and lutein (1.74) synthesis at the expense of violaxanthin (1.58), antheraxanthin (1.24), zeaxanthin (1.18) and neoxanthin (1.47) [277]. Increases are more pronounced in sugar beet when potassium is associated with an anion other than chloride [284]. It is thought that the higher levels noted in seedlings dusted with potassium iodide is due to a decrease in the oxidative destruction of the pigments [279].

#### (e) *Sulphur*

There is one report which indicates that a deficiency of sulphur reduces carotenoid production [109]; addition of sulphur (20 mg/kg) certainly increases the carotenoid levels of leaves of vetch, pea and lucerne in sand culture [285, 286].

#### (f) *Chloride*

Chloride has no effect on carotenoid levels in maize leaves [287] but salination reduces the levels in tomato leaves [288].



(g) *Micro-nutrients*

A summary of the main effects of micro-nutrients on carotenogenesis is given in Table 4.11. It is difficult to draw any general conclusions because the bases of many of the experiments are very different and interaction of the micro-nutrients has not yet been very seriously considered. For example, although excess calcium lowers carotenoid levels in lupin leaves this is reversed by K, Mg and B, K being the most effective [296]. Furthermore, the importance

Table 4.11 Effect of micro-nutrients on carotenogenesis in leaves

<i>Micro-nutrient</i>	<i>Plants</i>	<i>Effect</i>	<i>Ref.</i>
Aluminium	Forages	Reduces	289
Boron	Lucerne	Marked increase	290
	Maize	Increase after soaking in B.	291
	Cucumber	Optimum positive effect with 0.5 mg/kg soil	292
	Flax	Increases	293
Calcium } (lime) }	Clover	Increases; 0.5 mm particles best	294
	Lucerne	Increases 0.5 mm particles best	294, 295
	Lupin	Lowered by excess	296
	Timothy	Increases	295
Cobalt	Lucerne	Increases	297
Chromium	Maize	Increase in light but not in dark	298
Copper	Clover	Increases	299
	Sunflower	Increases at head-forming stage	300
	Tomato	Increases but inhibits at high levels	301
Iron	Lettuce	No effect	271
	Spinach	No effect	302
	Swiss chard	Reduces in deficiency	254
Maganese	Spinach	Increases	302
	Maize	Increases in light but not in dark	298
	Spruce ( <i>Pinus abies</i> )	Increases	303
Molybdenum	Clover	Increases	299
	Clover (red)	Increases on soils originally low	304
	Cucumber	Increases: optimum concentration 0.02 mg/kg soil	292
	Flax	Increases	293
	Pea	Increases	305
	Lucerne	Increases	306, 307
	Sunflower	Increases	308
Nickel	Lucerne	Increases	309
Titanium	Sugar beet	Increases	310
Vanadium	Lucerne	Increases	306
Zinc	Grape	Increases	311
	Sunflower	Increases, particularly at flowering stage	300

of magnesium:calcium balance in encouraging maximum carotenogenesis should not be overlooked; to take a specific case, in soya beans a high Ca:Mg ratio results in more carotenoid and less chlorophyll than normal and the situation is reversed on a high Mg:Ca ratio [312]. In a multi-trace element experiment cobalt and zinc appeared to be the most important for carotenogenesis in cucumber leaves and the further addition of copper, molybdenum, boron and manganese had little positive effect [313].

#### 4.7.5 PHYSICO-CHEMICAL PROPERTIES OF SOIL

In the case of oats and rye, soils with the following characteristics produced crops with high carotene levels: base-exchange capacity 20 mEq, total replaceable base 20 mEq, replaceable calcium 18 mEq, and replaceable magnesium 2 mEq, all per 100 g soil; loss on ignition 4%; nitrogen content 0.09%. Less than 15 mEq replaceable base/100 g and less than 0.08% nitrogen renders the soil unsatisfactory [272].

It has been claimed that increasing soil pH increases carotene production [233] and that in cress seedlings grown in water culture carotenogenesis is related to the pH of the cell sap [261]. Grasses on cultivated podzols have a higher carotene level than those grown on peat soils [314] but addition of mineral acids to peat soil improves the carotene levels in oats [315]. In both oats [316] and tomato [317] high soil moisture reduces the leaf carotenoid level although increasing moisture improves the carotene levels in Persian walnut leaves [318] and in maize, sunflower and cotton [319]. In early experiments drought *per se* was said to have little effect on carotenoid production [320] although recently it has been claimed that many plants accumulate carotenoids, particularly xanthophylls, under such conditions [321]. Leaves of *Pelargonium roseum* grown hydroponically contain more carotenoids than those grown in soil [322].

Experimentally imposed water stress, achieved by placing leaves in polyethylene glycol, results in lowering of carotenoid levels in leaves of wheat [347] and *Quercus robur* [322A], but in an increase in leaves of *Q. coccifera* and *Q. ilex* [322A].

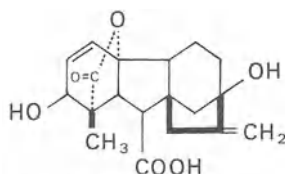
#### 4.7.6 ELEVATION

The carotene content of spinach varies with the elevation at which the crop is grown; maximum levels were observed at 400–600 m [323]. In general, however, increase in altitude results in lowered carotenoid levels [324] with relatively more epoxides being produced than carotenes [325]. Similarly, the carotene levels in leafy plants in the Apsheron peninsula were 2–3 times lower than in the Crimean peninsula [326] and the carotenoid levels in Siberian larch were lower in mountain locations than in the lowlands [327].

## 4.7.7 HORMONES

(a) *Gibberellic acid*

In a large number of experiments with seedlings of many different species an inhibition of carotenogenesis was noted after treatment with gibberellic acid (4.6) [328–338]. Observations which do not fit into this general picture include: (a) low concentrations inhibit whilst high concentrations stimulate carotenogenesis [339]; (b) carotene synthesis is stimulated whilst that of xanthophylls is not [340]; (c) no effect is apparent [341]; and (d) in potatoes there is a lowering effect only in virus-infected leaves [342].



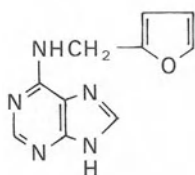
(4.6) Gibberellic acid

(b) *Abscisis acid*

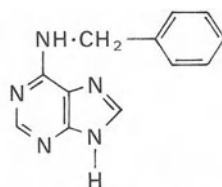
This hormone (2.11) decreases the carotene level in pea seedlings [343, 344] and in both isolated shoots [345] and intact seedlings of maize [346], as well as in greening etiolated barley [346] and wheat [347] seedlings.

(c) *Cytokinins*

An effect similar to that of abscisic acid has been observed with kinetin (4.7) (6-furfuryladenine), [336, 348–354]. Benzyladenine (4.8) however, stimulated synthesis in intact squash seedling cotyledons [355], in bean seedlings [356] and in excised barley leaves [357]. On the other hand, it did not inhibit destruction of carotenoids in plants kept either in light or darkness, although destruction of chlorophyll in the dark was inhibited [358]. Indeed, in the dark benzyladenine accelerated the decomposition of neoxanthin (1.47) [359].



(4.7) Kinetin



(4.8) Benzyladenine

(d) *Ethylene*

In shoot apices of etiolated pea seedlings ethylene completely inhibits the synthesis of carotenoids which is stimulated by light or phytochrome [344].

(e) *Indole acetic acid*

Carotene synthesis in maize leaves is reduced by indole acetic acid [360] but in the case of flax, stimulation is observed at the seedling and growing stage but little effect is observed at the flowering or later stages [361]. 2-Naphthyl acetic acid increases the amount of carotenoid in tobacco tissue cultures (Section 4.11) when calculated on a wet weight basis [362, 363] but not on a per cell basis [363].

## 4.7.8 PATHOGENIC AGENTS

(a) *Fungi*

In general the effect of fungal infection on leaf carotenoids is to reduce carotenoid levels in infected leaves (Table 4.12), although in some cases the effect is qualitative as well. For example, the pycnidial lesions on crab apple (*Malus ioensis*) leaves infected with common rust (*Gymnosporangium juniperi-virginiane*) contain relatively large amounts (34.5% of the total carotenoids) of  $\gamma$ -carotene (1.38); the unaffected portions of the leaves contain no  $\gamma$ -

Table 4.12 The effect of fungal infection on carotenoid levels in leaves

<i>Infecting organism</i>	<i>Host</i>	<i>Effect</i>	<i>Ref.</i>
<i>Colletotrichum graminicola</i>	Sorghum	Increased	364
<i>Eriophyes tiliae</i>	<i>Tilia cordata</i>	Complex: see text	365
<i>Erysiphe graminis</i>	Oats	Reduced	365
<i>Erysiphe</i> sp.	Pea	Reduced 23–36%	366
<i>Gloeocercospora sorghi</i>	Sorghum	Increased	363
<i>Gymnosporangium juniperi-virginiane</i>	<i>Malus ioensis</i> (crab apple)	Complex: see text	367
<i>Gymnosporangium sabinae</i>	Pear	Complex: see text	368
<i>Peronospora farinosa</i>	<i>Chenopodium nuttalia</i>	Reduced	369
<i>Plasmopara viticola</i>	Grape	Increased	371
<i>Polystigma rubrum</i>	Plum	See text	368
'Rust'	Clover	Reduced by 30%	371
<i>Uromyces pisi</i>	<i>Euphorbia cyparissias</i>	Increased for 10–15 days, then decreased	372
<i>Verticillium dahliae</i>	Cotton	Reduced	373
<i>Verticillium</i> sp.	Plum	Reduced	374

carotene [367] so a reasonable conclusion is that the pigment, which is a widespread fungal carotenoid (see Chapter 8), is made by the fungus itself. The same pattern is observed when *G. sabinae* parasitizes pear leaves.  $\alpha$ -Cryptoxanthin (4.1) and isocryptoxanthin (1.35) appear in galls of *Tilia cordata* infected by *Eriophyes tiliae* [365] and lycopene (1.11) appears in plum leaves infected with *Polystigma rubrum* [368].

The yellowing of box (*Buxus sempervivens*) leaves following parasitization with either fungi or insects is said to be due to excessive carotenogenesis [375] but this needs to be confirmed; the appearance might arise because of specific destruction of chlorophylls.

The gene pg<sup>11</sup> which confers oat stem rust resistance in adult oats is associated with lowered carotenoid levels; seedlings which have no additional resistance have normal carotenoid levels [376].

(b) *Virus infection*

No relationship has been found between carotene content and mosaic resistance factors in 18 varieties of tobacco [377] and carotene synthesis in sugar cane leaves is unaffected by sugar cane mosaic virus [378]. However, carotenoid levels in tomato leaves infected with tomato leaf curl virus were reduced [379] and a similar reduction was noted in *Brassica pekinensis* infected with turnip yellow mosaic virus [380, 381] and broad bean infected with mosaic virus [382].

(c) *Insect infection*

Table 4.13 demonstrates that a number of insect pathogens cause a reduction of carotenogenesis in leaves.

Table 4.13 The effect of insect infection on carotenoid levels in leaves

<i>Insect</i>	<i>Plant</i>	<i>Effect</i>	<i>Ref.</i>
<i>Empoasca fabae</i> (leaf hopper)	Lucerne	Reduces	382, 383
<i>Eutetranychus orientalis</i> (citrus brown mite)	Sour orange	Reduces	384
<i>Lopholeuocaspis japonica</i>	Mandarin orange	Reduces	385
<i>Panonychus ulmi</i> (red spider)	Apple	Reduces	386
<i>Stephanites pyri</i>	Pea	Reduces	387

(d) *Miscellaneous*


Carotenoid levels of sugar beet leaves are reduced when the plant is para-

sitized by dodder (*Cuscuta campestris*) [388]. Aflatoxin B, a fungal metabolite, sprayed on the seed bed reduces the carotenoid content of the cotyledons of germinating wheat seedlings by some 60% [389].

#### 4.7.9 HERBICIDES

Many herbicides when sprayed on leaves reduce carotenoid synthesis (Table 4.14). Others have interesting qualitative effects. Some, for example, inhibit

Table 4.14 Herbicides known to reduce carotenoid levels in plant leaves<sup>b</sup>

Herbicides	Plant	Ref.
ALAR	Radish <sup>a</sup>	390
2,4-D	Bean <sup>b,c</sup>	391, 392
Diuron	Apple	394, 393
(2,4-Dichlorophenoxy acetic acid)		
Maleic hydrazide	Walnut	394
Paraquat ( $10^{-4}$ M)	Flax <sup>a</sup>	395
Phosphon D	Radish	396
Prometryne	Onion <sup>d</sup>	397
Pyrazone	Various	398
(1-Phenyl-4-amino-6-chloro-6-pyridazinone)		
Pyrichlor	Various	399
(2,3,5-Trichloropyridine)		
Sandoz 6706	Wheat <sup>b</sup>	400
Sandoz 9789	Wheat <sup>b</sup>	400
Sandoz 9789	Mustard <sup>g</sup>	400A, 501
Simazine (0.5 kg/ha) <sup>e,f</sup>	Wheat <sup>b</sup>	401
Solan:  -NHCOCH (Me)Pr	Carrot	403
Terbacil	Apple	393

#### Notes

<sup>a</sup> Isolated leaf discs

<sup>b</sup> Seedlings

<sup>c</sup> Cotyledons

<sup>d</sup> Only under drought conditions; in moist conditions no effect

<sup>e</sup> Simazine (and linuron) have no effect on maize plants [402]

<sup>f</sup> Stimulates in leaves of two-year-old apple trees [393]

<sup>g</sup> Specific effect on carotenoids

<sup>h</sup> See also [500]

cyclization of the acyclic precursor lycopene which results in its accumulation at the expense of  $\beta$ -carotene (1.13) and other cyclic carotenoids. In other cases the biosynthetic intermediates between phytoene (1.36) and lycopene (1.11), that is, phytofluene (1.37),  $\zeta$ -carotene (1.14) and neurosporene (2.14) (see Chapter 2), also accumulate. Herbicides with these effects include amino-triazole [390], pyrichlor [403], CCC [404] (although it is said to have no effect on wheat seedlings [405]), nicotine [406] and CPTA [407]. Sandoz 6706 inhibits the desaturation of phytoene which accumulates in large amounts

[403] partly in the form of its 1,2-oxide [408]. Dichloromate is interesting in that it appears to inhibit the desaturation at the  $\zeta$ -carotene  $\rightarrow$  neurosporene stage as the former accumulates in treated plants [403].

A few herbicides tend to increase carotenoid levels in leaves [409, 410]; in the case of various propionic acid derivatives (e.g. 3-chloropropionic acid) this is said to be due to the inhibition of lipoxidase (Section 4.10.3) which results in the slowing down of the oxidative destruction of carotenoids [411]. There is one report of increased carotenoid levels on treatment with 2,4-D [412], although a number of previous reports to the contrary are indicated in Table 4.14.

PRB-8 ( $\alpha$ -chloro- $\beta$ -(3-chloro-*p*-toluyl)propionitrile) inhibits the phytochrome-stimulated carotenogenesis in leaves [413].

In both jack beans and potatoes, spraying with CCC causes a rapid drop of up to 50% in carotenoid levels which take three weeks to recover [414, 415]. CMH (N,N-dimethyl-(2-chloroethyl) hydrazonium hydrochloride) increases the levels in wheat seedlings but they return to normal in mature plants [409].

Although the information given in Table 4.14 and that given above reflects the generally accepted situation, the varying conditions under which the herbicides are applied must clearly be appreciated if difficulties of interpretation are to be avoided.

#### 4.7.10 FUNGICIDES AND INSECTICIDES

The fungicide cuprosan reduces carotenoid levels in grasses [416] whereas in cabbage the effect of DDT depends on the previous fertilizer treatment; with superphosphate levels drop whilst with ammonium or nitrate they rise [417].

#### 4.7.11 ANTIBIOTICS

A number of antibiotics have been tested for their effect on carotenogenesis (Table 4.15).

#### 4.7.12 OSMOTIC PRESSURE

Detached wheat leaves stressed by negative ( $-7$  bars) osmotic pressure prior to illumination exhibit reduced carotenoid synthesis on illumination. This is overcome by treatment with N<sup>6</sup>-benzyladenine plus gibberellic acid [346].

### 4.8 BIOSYNTHESIS

The general pattern of biosynthesis of carotenoids is covered in Chapter 2, the main problem in leaves is the site or sites of synthesis. The pigments

Table 4.15 The effect of some antibiotics on carotenoid levels in green leaves

<i>Antibiotic</i>	<i>Plant</i>	<i>Effect</i>	<i>Ref.</i>
Actinomycin D	Cotton – cotyledons	Increases	418
	Bean – primary leaves	Reduces	419
Chloramphenicol	Cotton – cotyledons	Increases	418
	Bean – primary leaves	Reduces	419
Cycloheximide	Cotton – cotyledons	Increases	418
	Bean – primary leaves	Reduces	420
Streptomycin	Bean	Reduces	421
	Radish	Reduces	422
Tetracycline	Bean	Reduces	421
	Radish	Reduces	422

are confined to the chloroplasts and there is still discussion as to whether this organelle can make carotenoids and other isoprenoids *de novo* [423]. This section focuses on the two main questions of whether the chloroplast can synthesize the fundamental precursor, acetyl-CoA, and convert it into mevalonic acid, the first specific precursor of terpenoids. The further steps to carotenoids, which have been discussed in detail in Chapter 2, can be carried out by isolated chloroplasts suitably prepared [434] and by partly purified preparations therefrom [425].

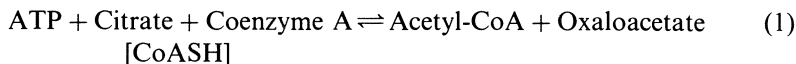
If chloroplasts are autonomous, then they must be able to synthesize the enzymes necessary to carry out the various steps in carotenogenesis. There is no doubt that they have the ability to synthesize protein [426] but the number of proteins they can synthesize is limited and many chloroplast proteins must be assembled outside the organelle and transported thereto at sometime during its development [427]. The fact that chloroplasts can accumulate and synthesize proteins leads to the question whether they contain all or some of the enzymes concerned with carotenogenesis.

#### 4.8.1 ACETYL-COA FORMATION

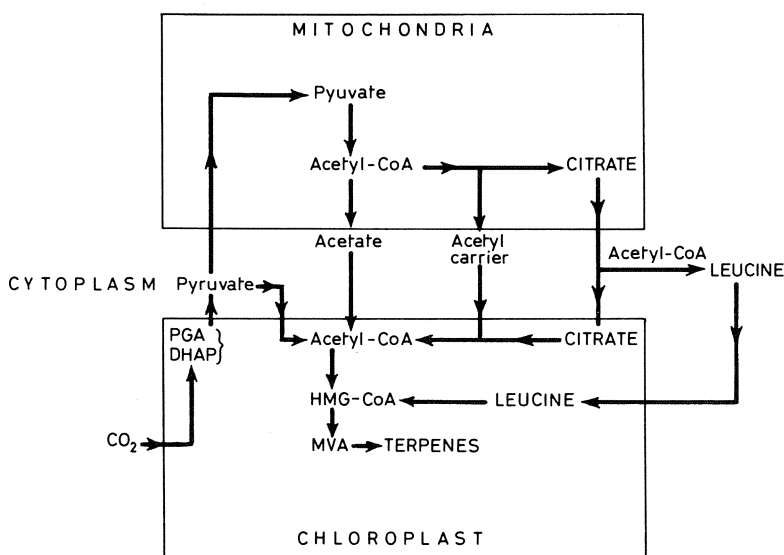
Acetyl-CoA is normally synthesized either from pyruvate by the action of pyruvate dehydrogenase, pyruvate itself being formed by glycolysis, or by  $\beta$ -oxidation of fatty acids. Neither glycolysis nor oxidative breakdown of fatty acids has yet been shown to occur in chloroplasts [see 427A] so one is led to the view that acetyl-CoA is formed either outside the chloroplast and transported there or inside by an unconventional route. If one assumes that acetyl-CoA is formed in leaf mitochondria, then five possibilities of transport into the chloroplast exist: (i) direct entry as acetyl-CoA; (ii) entry as acetate followed by activation within the chloroplast; (iii) transport as acetyl L-carnitine as in animals; (iv) conversion into citrate by condensation with oxaloacetate in the mitochondria followed by transport of the citrate to the chloroplast



where it could be cleaved by the enzyme ATP-citrate lyase (Equation 1); and (v) conversion into an amino acid (valine or leucine) which could be transported into the chloroplast and there converted into hydroxymethylglutaryl-CoA the immediate precursor of mevalonic acid. Possibility (i) appears to be ruled out because acetyl-CoA is not used for fatty acid synthesis by isolated



chloroplasts although acetate in the presence of the appropriate co-factors is [428]. Possibility (ii), hydrolysis of acetyl-CoA by acetyl-CoA hydrolase, which is present in plants, transfer of free acetate to the chloroplast and its conversion there back into acetyl-CoA by acetyl-CoA synthetase, is an attractive idea. However, free acetate has not yet been detected in plants, but the synthetase is very active in chloroplast preparations [428]. Acetyl L-carnitine (possibility iii) has been observed only spasmodically in plants and for (iv) to have any credence a citrate lyase must be present in chloroplasts. Such an enzyme has not been unequivocally demonstrated in chloroplasts although slight activity is always associated with carefully prepared chloroplasts [429]. In the final possibility (v), acetyl-CoA could be converted into the carbon skeleton of leucine in the cytoplasm [430]; after its formation by transamination, leucine could move freely into the chloroplast where it could yield hydroxymethylglutaryl-CoA. It is known that in fungi, for example, leucine is extremely carotenogenic (Chapter 2).



**Fig. 4.5.** Possible metabolic collaboration between mitochondria, cytoplasm and chloroplasts to produce carotenoids within the chloroplasts.

A further possibility is that pyruvate formed in mitochondria is shuttled into the chloroplast where it is converted into acetyl-CoA by pyruvate dehydrogenase. The clear demonstration of this enzyme in the chloroplast has not yet been reported [431] although in some experiments pyruvate is a good substrate for carotenoids in isolated chloroplasts [432]. Clearly the source of acetyl-CoA in chloroplasts is not yet settled but Fig. 4.5 summarizes the possibilities which have just been discussed, and it must be remembered that it is claimed that isolated chloroplasts cannot convert  $\text{CO}_2$  into acetyl-CoA [433]; on the other hand, spinach chloroplasts effectively incorporate  $^{14}\text{CO}_2$  into fatty acids [434].

The possibility that pyruvate can be synthesized directly in the chloroplast from  $\text{CO}_2$  has some support and a number of experiments suggest that the immediate source could be serine which can be formed from  $\text{CO}_2$  directly (Fig. 4.6) via glycolate [435]. One objection to this view, that pyruvate kinase required to convert phosphoenolpyruvate into pyruvate is not present in chloroplasts [436] has been challenged [437]. However, it is now known that the reactions outlined in Fig. 4.6 involve the collaboration of chloroplasts, peroxisomes and mitochondria.

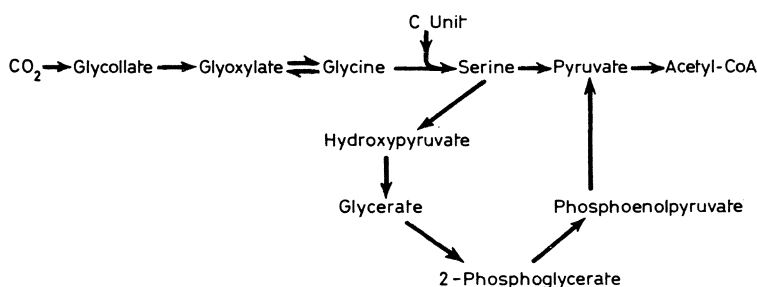


Fig. 4.6. A possible route for conversion of  $\text{CO}_2$  into acetyl-CoA.

#### 4.8.2 FORMATION OF MEVALONIC ACID

The next question is whether the chloroplast can convert acetyl-CoA, however it is formed, into mevalonic acid. The accepted pathway is outlined in Fig. 4.7 (see also Chapter 2). The only experimental evidence available is the demonstration of hydroxymethylglutaryl-CoA (HMG-CoA) reductase activity in pea chloroplasts [438]. The need for the demonstration in the chloroplast of the earlier enzymes in the sequence is clearly great; until this is done the possibility remains that HMG-CoA (from leucine) and not acetyl-CoA is provided extra-plastidically.

If the mevalonic acid used for carotenogenesis in the chloroplast is produced from pyruvate or acetyl-CoA formed in mitochondria, there must be a very rapid two-way transport between the two organelles. In greening tissue  $^{14}\text{CO}_2$  is more effectively incorporated into chloroplasts than is exogenous  $[2\text{-}^{14}\text{C}]$  mevalonic acid [426, 439].

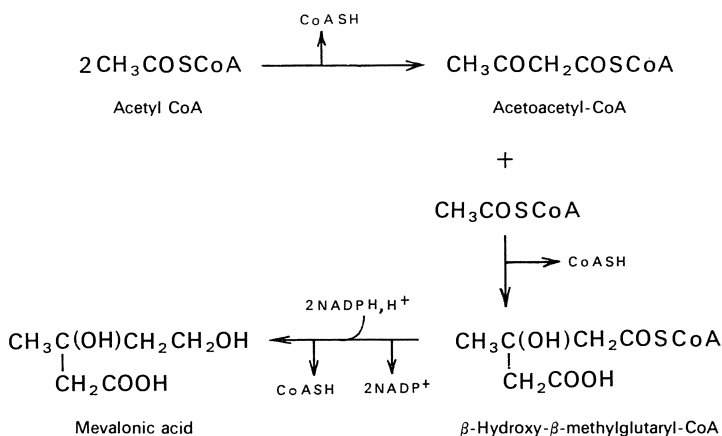


Fig. 4.7. Conversion of acetyl-CoA into mevalonic acid.

#### 4.8.3 CONVERSION OF MEVALONIC ACID INTO CAROTENOIDS

The evidence for the conversion has already been given. All that needs to be emphasized here is that a number of the enzymes involved are under nuclear control (Fig. 4.8) [440] and must have been synthesized outside the chloroplast and transported there either during or after the formation of the organelle.

#### 4.8.4 CONCLUSION

It must be concluded that, although carotenoids are present exclusively in the chloroplast, present evidence suggests that not all the steps in the bio-

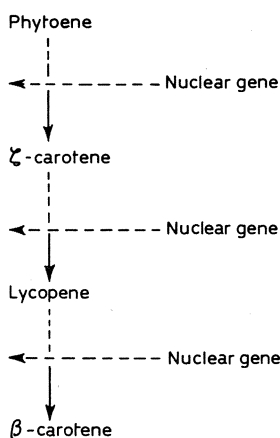


Fig. 4.8. Nuclear control of carotenoid synthesis in higher plants.

synthetic pathway take place there. If the basic building unit is pyruvate or acetyl-CoA it probably arises in the mitochondria but hydroxymethylglutaryl-CoA may be the first compound involved in the chloroplast. Many of the enzymes involved in the late stages are probably synthesized outside the chloroplast.

#### 4.9 REGULATION OF SYNTHESIS

It is apparent from studies on greening etiolated seedlings that there is a stimulation of carotenogenesis associated with the development of functional chloroplasts from etioplasts (Section 4.7.1(a)). The mechanism by which light stimulates the formation of chloroplasts is far from being fully understood but it is clear from the tight coupling of carotenoid synthesis within the system that it is under the same 'master' control.

A more specific problem is how light channels terpenoid synthesis from the extra-plastidic synthesis of sterols, ubiquinone and related compounds to the plastidic synthesis of carotenoids, phytol, plastoquinone, etc. One view [441, 442] is that it is a result of enzyme segregation and specific membrane permeability. The basic assumption (Fig. 4.9) is that both extra-chloroplastidic and chloroplastidic sites of synthesis have a common 'backbone'

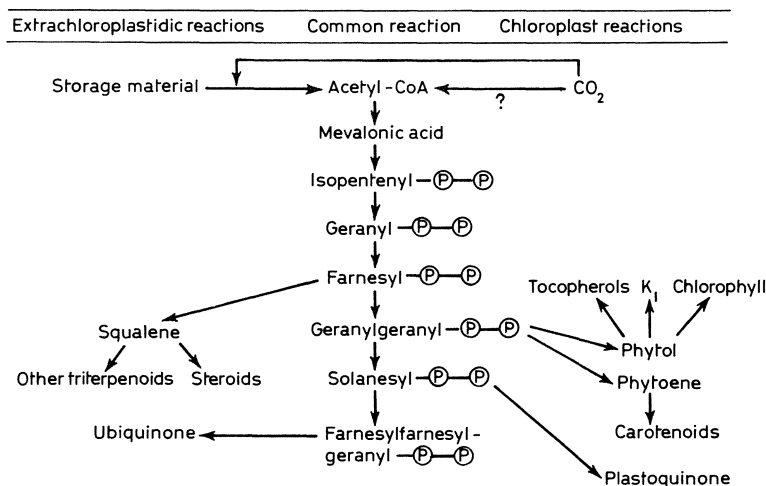


Fig. 4.9. General pattern of terpenoid synthesis in higher plants.

of biosynthesis. In addition, each compartment has specific enzymes which act on certain terpenoid pyrophosphates. For example, farnesyl pyrophosphate ( $C_{15}$ ) can be converted into geranylgeranyl pyrophosphate ( $C_{20}$ ) at both sites. However, the former can be converted into squalene (triterpene and sterol precursor) only outside the chloroplast whilst the  $C_{20}$  compound can be converted into phytoene (carotenoid precursor) only within the

chloroplast [426, 439]. The second assumption, which has recently been demonstrated experimentally [439A], is that the chloroplast is relatively impermeable to mevalonic acid and thus etiolated seedlings cannot use the mevalonic acid formed in the cytoplasm for carotenoid synthesis in the dark in etioplasts, but use it to form sterols required for membrane synthesis. On illumination, chloroplasts begin to develop and to fix  $\text{CO}_2$  *inter alia*, into mevalonic acid in the chloroplast; this cannot pass out of the chloroplast and thus is available specifically for synthesis of chloroplast terpenoids. The main evidence in support of this view is that  $^{14}\text{CO}_2$  is more effectively incorporated into carotenoids than is  $[2\text{-}^{14}\text{C}]$  mevalonic acid in greening seedlings, whilst the reverse is the case with sterol synthesis [426, 439]. The validity of this view cannot be fully assessed until the primary source of mevalonic acid in the chloroplast, discussed in the previous section, is decided. It may be that activation of key enzymes by light is more important than permeability.

In mature chloroplasts, carotenoid levels remain constant so that a precise regulation appears by which carotenoid synthesis either stops or is balanced by destruction. In mature chloroplasts from young leaves,  $^{14}\text{CO}_2$  uptake into carotenoids is very limited [442] which suggests that turnover is sluggish if it occurs at all. However, turnover has been claimed in blackcurrant leaves: 50 days after exposure of the leaves to  $^{14}\text{CO}_2$  the specific activity of the carotene fraction has dropped by some 60 times [443].

## 4.10 METABOLISM

### 4.10.1 FORMATION OF ABSCISIC ACID

Absciscic acid (2.11) which promotes senescence and abscission of leaves can be considered to be a degraded carotenoid and this possibility is enhanced by the fact that it has the same chirality at C-6 as has violaxanthin (1.58). However, there is no direct evidence that violaxanthin is converted into absciscic acid *in vivo*, but photo-oxidation of violaxanthin and neoxanthin (1.47) *in vitro* yields compounds (e.g. xanthoxin) with absciscic acid activity [444]. Similar activity is produced by oxidizing  $\beta$ -carotene by lipoxidase in the presence of unsaturated fatty acids [445]. A recent observation that lysed chloroplasts from avocado can form absciscic acid from mevalonic acid [446] supports the view that it need not necessarily be formed from carotenoids because chloroplasts normally are poor in converting exogeneous mevalonic acid into carotenoids (see previous section).

### 4.10.2 SENESCENCE

In his early classical experiment Tswett found that by far the major fraction of carotenoids in senescing (autumn) leaves behaved as carotenes in phase

tests, however, they could be separated chromatographically from true carotenes and he suggested the name 'autumn xanthophylls' [447]. Palmer [448] proposed the name 'autumn carotenes' but eventually it was shown that they were the xanthophylls originally present which had become esterified with long chain fatty acids [449]. At one stage they had been considered oxidation products of normal plastid carotenoids [450]. There is now agreement that in leaves which show this phenomenon, that is, those from deciduous trees, carotenes disappear more quickly than xanthophylls so that the carotene:xanthophyll ratio drops compared with the ratio in green leaves [451–454] (see also [455] for a review). The esterified xanthophylls are present in the cell sap and not in the chloroplasts [451]; presumably when the plastids disintegrate they liberate their pigments into the cytoplasm where they are rapidly esterified.

The pattern in senescing leaves of tobacco plants is, however, different from that in deciduous trees [456, 457];  $\beta$ -carotene survives longer than do the xanthophylls and no esterified xanthophylls are formed [458]. In contrast, when barley plants are blanched by placing them in darkness carotenes disappear before xanthophylls [459] and neoxanthin becomes the main component [460]. Early reports that carotenoids of leaves which are normally yellow (e.g. young leaves of *Euonymus japonica* or the *aurea* varieties of *Sambucus nigra*) [461] and those of the autumn leaves of tropical evergreens [462] are typically 'autumn carotenoids' need reassessment with modern techniques.

#### 4.10.3 DEGRADATION *IN VIVO*

Although carotenoid levels in mature leaves remain relatively stable until the onset of senescence, systems which will destroy the pigments can be extracted from such leaves. Two systems are, for example, present in sugar beet leaves, one located within the chloroplast (optimum pH 7.5 with crocetin (1.27) as substrate) [463] and one within the mitochondrion (optimum pH 3.5) [464–467]. The mitochondrial preparation can be separated into soluble and insoluble enzymes by treatment with Triton X-100. The soluble system has properties of a haemoprotein-lipoxidase/lipid complex [464, 465]. The particulate fraction is a peroxidase system which requires both oxalate and a thermostable factor but not lipids as co-factors [466, 467]. Peppers contain a carotenoid-destroying system [468] and purified lipoxxygenase from peas bleaches carotenoids [469], as does soya bean lipoxxygenase in the presence of linoleic acid [470]. This confirms the pioneer work of Sumner and Dounce on lipoxxygenase (see first edition of this book).

If seedlings of barley (*Hordeum vulgare*) [460], potato and maize [471] germinated in the light are placed in darkness then the carotenoids disappear relatively quickly. The pattern of change in barley, indicated in Fig. 4.10, emphasizes a short burst of synthesis before all the pigments except

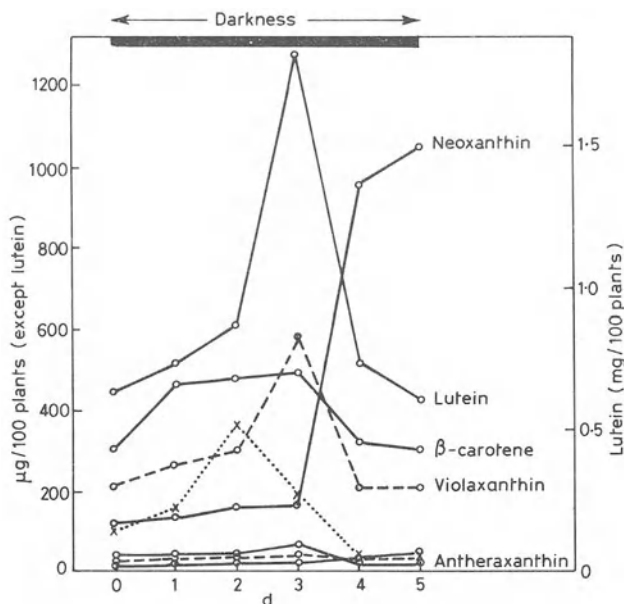


Fig. 4.10. Destruction of carotenoids in barley seedlings grown in the light and then placed in darkness [redrawn from 460].

neoxanthin (1.47) disappear [460]. The accumulation of neoxanthin has been explained by assuming that it is the common oxidation product of all other carotenoids present; this explanation can be challenged. Cobalt (0.1–1.0% cobalt sulphate) will inhibit the destruction of carotenoids in normal barley leaves placed in the dark [472]. The same effect is observed in wheat seedlings with  $\text{Co}(\text{NO}_3)_2$  and  $\text{MgSO}_4$  (0.6%) [473]. Rapid loss of pigments in darkness does not occur in leaves of some trees; for example, in leaves of black locust (*Robinia pseudoacacia*) and spruce (*Picea excelsa*) carotenoids are stable for at least 40–80 hours in the dark [471].

Leaves of thermophilic plants illuminated at low temperatures ( $1^\circ\text{C}$ ) lose both their carotenoids and chlorophylls by what appears to be a photo-oxidative process mediated by singlet oxygen [474] (see also Chapter 3).

#### 4.11 PLANT TISSUE CULTURES

Cultures of meristematic tissue of rose (Paul's Scarlet) contain little or no  $\beta$ -carotene or lutein, but relatively high concentrations of oxidized  $\beta$ -carotene derivatives such as violaxanthin, auroxanthin and neoxanthin [475]. A quantitative comparison of the carotenoids in leaf and tissue cultures is given in Table 4.16. 'Colourless' callus cultures of *Kalanchoe cruenta* have a very similar carotenoid content [476] although with other plants the amounts present are lower [477]. Carotenoids are also present in *Petunia* tissue culture

[477, 478]. In contrast, the carotenoids in green callus cultures are very similar to those in green leaves and those in callus cultures of *Ruta graveolens* are also qualitatively the same as in leaf [479]. Carotenoids, including two unidentified pigments [483], were also present in tissue culture of flax endosperm [480]. 'Dark' tissue cultures of tobacco contain some 2–3 times more carotenoids than do 'colourless' calluses [481]. Cultures of *Tanacetum vulgare* contain only xanthophylls [482]. Naphthylacetic acid increases the amount of carotenoids in tobacco tissue culture [484] but this is only apparent on a wet weight basis, for no effect is observed when the amount is calculated per  $10^6$  cells [485]. Gibberellic acid increases the carotenoid levels in sugar beet tissue when the tissues are transferred from darkness to light or are under continuous illumination [486]. Root fragments of endive (*Cychorium intybus*) if treated with ethylene or kept under anaerobic conditions for a short time at the beginning of the culture period, will eventually (one month later) yield tissues low in carotenoids [487].

Table 4.16 Comparative carotenoid distribution in rose tissue cultures and leaves [475]

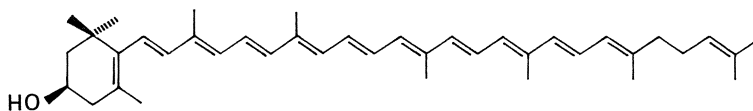
Pigment	Concentration $\mu\text{g/g}$ dry wt.	
	Tissue culture	Leaf
$\beta$ -Carotene (1.13)	0.0	96.3
Zeaxanthin (1.18)	1.07	4.3
Antheraxanthin (1.24)	0.0	3.6
Lutein (1.74)	0.0	176.7
Violaxanthin (1.58)	3.69	0.0
Auroxanthin (1.62)	2.49	0.0
Neoxanthin (1.47)	2.77	19.0
Total	10.02	299.9

#### 4.12. PARASITIC AND SAPROPHYTIC PLANTS

The yellow-orange colour of dodders is caused by a relatively high concentration of carotenoids. In the Californian marsh dodders *Cuscuta salina* and *C. subinclusa*,  $\gamma$ -carotene (1.38) is the major carotene present, occurring to about twice the extent of  $\beta$ -carotene (1.13); traces of lycopene (1.11) and rubixanthin (4.9) were also present [488]. In *C. australis* detached from its host (*Medicago sativa*),  $\beta$ -carotene is at a somewhat higher concentration than  $\gamma$ -carotene and  $\beta$ -carotene-5,6-epoxide and lutein (1.74) are also present [489]. Carotene also predominates in dodder parasitizing sugar beet and it is accompanied by violaxanthin and lutein [490]. *Viscum album* (mistletoe) from various hosts contains the same carotenoids as do host leaves, but with rather more  $\alpha$ -carotene than  $\beta$ -carotene [491]. The saprophytes, *Corallorrhiza*



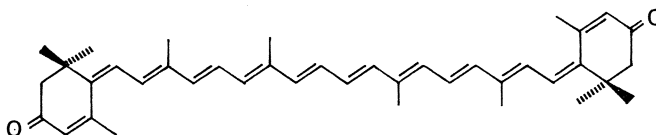
*innata*, *Epipogium aphyllum* and *Monotropa hypopitys* contain the usual leaf carotenoids in rather lower amounts and with relatively more lutein than  $\beta$ -carotene [492]. The saprophytic orchid *Neottia nidus-avis* contains the expected carotenoids in its chloroplasts [493].



(4.9) Rubixanthin

#### 4.13 AQUATIC HIGHER PLANTS

Rhodoxanthin (4.10) was isolated from *Potamogeton natans* in 1893 by Monteverdi, who in collaboration with Lubimenko isolated it in crystalline form in 1913, (see 494 for details). Much more recent work on *P. perfoliatus* has indicated the presence of carotene, lutein, violaxanthin and neoxanthin as well as three unidentified pink pigments, none of which was rhodoxanthin [495].



(4.10) Rhodoxanthin

Eloxanthin, isolated from the Canadian pondweed *Elodea canadensis* [494], has now been shown to be lutein 5,6-epoxide; lutein is also present [496].

Shaded leaves of *Potamogeton fluitans* are green but turn red on exposure to strong light; this is claimed to be due to the replacement of chlorophyll in the chloroplasts by carotenoids. The change is reversed when the light source is removed [497]. Flowering of *Lemna minor* growing on Hunter's medium is inhibited by addition of carbohydrate; at the same time carotenoid synthesis is also inhibited [498]. Carotenoid synthesis by the water plant *Phragmites communis* is stimulated by the addition of alanine and glutamate to the medium; tryptophan is without effect [499].

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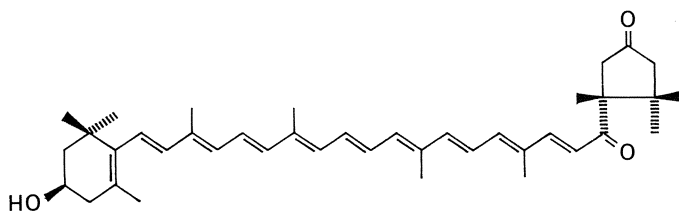
# CAROTENOIDS IN HIGHER PLANTS

## 5.1 REPRODUCTIVE TISSUES

### 5.1.1 ANTHERS, POLLEN AND FILAMENTS

#### (a) *Anthers*

Antheraxanthin (1.24) together with a *cis*-isomer, possibly an artefact, was first isolated from the anthers of *Lilium tigrinum* [1–3]. It is now evident, however, that antheraxanthin is a widespread carotenoid and is neither confined specifically to anthers, nor always present in pollens which contain carotenoids. The known carotenoid distribution in anthers is given in Table 5.1. In those cases where a comparison has been made between the carote-



(5.1) Capsanthone

noids of leaves and petals and anthers, the carotenoids of the last named are revealed as moderately complex mixtures. *Delonix regia* anthers, for example, contain ten pigments (with zeaxanthin representing 90% of the total) whilst its petals contain twenty-nine (see Table 5.4) [7]. Of particular interest is the appearance in some anthers of capsanthin (1.30), once considered a pigment unique to red peppers (Section 5.1.4), and a diketo carotenoid, 4-keto-capsanthin. The appendices of the anthers of *Typhonium divaticatum* and *Arum maculatum* contain mainly epoxides, with  $\alpha$ -carotene-5,6-epoxide predominating, together with smaller amounts of  $\alpha$ -carotene (1.12) and traces of  $\beta$ -carotene (1.13); the pigments are present in the chromoplasts of the tissues [14].

Table 5.1 Carotenoid composition of anthers

Species	Carotenoids	Ref.
<i>Aesculus rubicunda</i>	1, 2, 3, 4, 5	4
<i>Allium</i> spp.	1 <sup>h</sup>	26
<i>Clivia miniata</i>	1, 6, 7	5
<i>Colchicum autumnale</i>	1, 7, 8 <sup>a</sup>	6
<i>Dahlia</i> spp.	9	6
<i>Delonia regia</i>	10, 11, 12, 13, 14 <sup>b</sup>	7
<i>Lilium</i>	11 <sup>c</sup>	8
<i>L. leichinii</i> var. <i>maximowiczii</i>	2, 5	8A
<i>L. davidii</i> var. <i>willmottiae</i>	2, 5	8A
<i>L. maxime</i>	2, 11 <sup>d</sup>	6
<i>L. regale</i>	2, 11 <sup>d</sup>	6
<i>L. tigrinum</i>	2, 11 <sup>d</sup>	1, 2, 6
<i>L. umbellatum</i>	1, 2	6
<i>L. willmottiae</i> <i>unicolor</i>	1, 2	6
<i>Narcissus poeticus</i>	1, 6, 8, 11, 15, 16, 17 <sup>d, e</sup>	9
<i>N. pseudonarcissus</i>	1, 7, 8, 11, 15, 16, 17 <sup>f</sup>	9
<i>N.</i> spp.	1, 6, 8 <sup>d</sup>	10, 11
<i>Rosa</i> spp.	1, 14, 15, 17 <sup>g</sup>	12
<i>Tropaeolum majus</i>	1, 6, 7, 8, 11, 12, 18, 19, 20	13
<i>Tulipa</i> spp.	1, 8, 11, 15, 16, 17	7, 9
<i>Zea mays</i>	1, 6 + xanthophylls	13a

## Key

1. $\beta$ -Carotene	(1.13)	11. Antheraxanthin	(1.24)
2. Capsanthin	(1.30)	12. $\beta$ -Cryptoxanthin	(1.60)
3. Capsanthone	(5.1)	13. Violaxanthin	(1.58)
4. 4-Ketocapsanthin		14. Auroxanthin	(1.62)
5. Capsorubin	(1.59)	15. $\beta$ -Carotene 5,6-epoxide	
6. $\alpha$ -Carotene	(1.12)	16. $\beta$ -Carotene 5,6-5'6'-diepoxide	
7. Lutein 5,6-epoxide		17. Flavoxanthin + Chrysanthemaxanthin	(4.3) (4.2)
8. Lutein	(1.74)	18. $\alpha$ -Cryptoxanthin	(4.1)
9. Lycopene	(1.11)	19. $\delta$ -Carotene	(4.4)
10. Zeaxanthin	(1.18)	20. $\gamma$ -Carotene	(1.38)

## Notes

<sup>a</sup>Lutein present as esters.<sup>b</sup>Zeaxanthin major pigment.<sup>c</sup>Major pigment *cis*-antheraxanthin.<sup>d</sup>Also *cis*-antheraxanthin.<sup>e</sup>Also isomer of (1.38).<sup>f</sup>Also  $\beta$ -zeacarotene (2.12).<sup>g</sup>Also unspecified epoxides.<sup>h</sup>Spatial isomers.

## (b) Pollen

Carotenoids (lipochromes) were first noted in pollen of *Verbascum thapsiforme* in 1892 [15] and in the early 1940s they were detected but not characterized in pollen from white willow (*Salix alba*), black willow (*S. nigricans*), *Lilium candidum* and *Taraxacum officinale* [16]. The presently known qualitative distribution in pollen is given in Table 5.2. Carotenoids have also been observed in apple and plum pollen [17] but they appear to be present only in traces or absent from the pollen of *Pinus radiata* and *P. ponderosa* [21], *Typha latifolia* [22], *Populus nigra*, *Pinus montana*, *Betula pubescens*, *Campanula persicifolia* [16], *Alnus glutinosa*, pear [23] and *Tilia platyphyllos* [6]. No vitamin A-active pigments were found in the pollen of *P. densiflora* and *P. thunbergi* [24]. Sterile pollen from *Allium cepa* contains less carotenoid (1.61–1.79 mg/100 g fresh tissue) than fertile pollen (2.76–3.0 mg/100 g fresh weight) [25, 26] but the biological significance of this has still to be assessed. Sporopollenin, the highly resistant macromolecule of the outer wall of pollen, is considered to be a carotenoid polymer [27].

Table 5.2 Carotenoid components of pollen

Species	Pigments	Ref.
<i>Acacia dealbata</i>	1, 2, 3, 4, 5, 6	17
var. <i>legaulois</i>		
<i>Aesculus rubicunda</i>	2, 7, 8, 9, 10	4
<i>Aster</i> spp. <sup>a,b</sup>	1, 6	18
<i>Chrysanthemum</i>	1, 2, 6	6
<i>cinerariaefolium</i> <sup>b,c</sup>	6	19
<i>Helianthus annuus</i> <sup>a,b</sup>	1, 2, 6	
<i>Helianthus tuberosus</i> <sup>a,b</sup>	1, 2, 6	6
<i>Pinus radiata</i>	2, 4, 6, 11, 12	20
<i>Sonchus</i> sp.	2	20A
<i>Tropaeolum majus</i>	1, 2, 4, 6, 13, 14, 15, 16, 17	13
<i>Zea mais</i> <sup>a</sup>	1, 2, 6	6

## Key

- |                                   |                                   |
|-----------------------------------|-----------------------------------|
| 1. $\alpha$ -Carotene (1.12)      | 10. 4-Ketocapsorubin              |
| 2. $\beta$ -Carotene (1.13)       | 11. Violaxanthin (1.58)           |
| 3. $\alpha$ -Carotene-5,6-epoxide | 12. Neoxanthin (1.47)             |
| 4. Lutein 5,6-epoxide             | 13. $\delta$ -Carotene (4.4)      |
| 5. Flavoxanthin (4.3)             | 14. $\gamma$ -Carotene (1.38)     |
| 6. Lutein (1.74)                  | 15. $\alpha$ -Cryptoxanthin (4.1) |
| 7. Capsanthin (1.30)              | 16. $\beta$ -Cryptoxanthin (1.60) |
| 8. Capsorubin (1.59)              | 17. Antheraxanthin (1.24)         |
| 9. Capsanthone (5.1)              |                                   |

## Notes

<sup>a</sup> The carotenes were not separated.

<sup>b</sup> Lutein present as esters.

<sup>c</sup> *cis*-isomers also present.



(c) *Filaments*

The filaments of flowers of *Delonia regia* and *Narcissus* spp. have been compared in carotenoid content with the corresponding anthers. Small but significant differences have been noted and this is indicated for *N. poeticus* in Table 5.3.

Table 5.3 Comparison of carotenoids in anthers, filaments, styles and stigmas in *Narcissus poeticus* (9) (values are % of total carotenoids).

Pigment	Anthers	Filaments	Styles and stigmas
$\alpha$ -Carotene (1.12)	2.0	—	—
$\beta$ -Carotene (1.13)	60.6	10.1	39.5
$\beta$ -Carotene-5,6-epoxide	4.2	2.9	—
$\beta$ -Carotene-5,6,5',6'-diepoxide	17.3	10.2	—
Lutein-5,6-epoxide	—	15.2	20.9
Antheraxanthin (1.24)	6.9	—	3.2
Lutein (1.74)	1.7	53.9	31.7
Chrysanthemaxanthin (4.2)	7.3	7.7	1.1
Unknown	—	—	3.6
Total Carotenoids mg/g (dry wt.)		0.3	4.9

(d) *Styles and stigmas*

The female parts of flowers have only been examined for *Narcissus* spp. and *Tulipa* spp. [9]. Significant differences, both quantitative and qualitative, have been observed when compared with anthers and filaments as indicated for *N. poeticus* in Table 5.3. Analyses of combined anthers and styles of four *Rosa* spp. have been reported [28].

## 5.1.2 FLOWERS

(a) *Qualitative distribution*

Not all flower petals contain carotenoids but if they do the pigments accumulate in developing chromoplasts very much as they do in maturing carotenogenic fruit (Section 5.1.4c). Usually chlorophylls disappear during the development of the chromoplasts but in some species, e.g. *Caltha palustis* and *Forsythia intermedia*, the mature flowers still contain chlorophyll.

From the point of view of carotenoids, flowers may be separated into three main groups which contain: (i) highly oxidized xanthophylls, especially 5,8-epoxides such as auroxanthin (1.62) and flavoxanthin (4.3); (ii) large amounts of carotenes such as  $\beta$ -carotene (1.12) and lycopene (1.11); and (iii) highly species-specific pigments such as eschscholtzxanthin (1.25) in *Eschscholtzia*

*californica*. The qualitative distribution of carotenoids in flowers already examined is given in Table 5.4. It will be apparent that modern investigations have revealed a far more complex picture than did some earlier investigations; there is no reason to believe that a re-examination of other earlier work would not reveal a similarly complex situation.

Table 5.4 Carotenoid distribution in flower petals

Species	Pigments	Ref.	Notes
<i>Acacia decurrens</i> var. <i>mollis</i>	1, 2	29, 30	
<i>A. discolor</i>	1, 2	29	
<i>A. linifolia</i>	1, 2	29	
<i>A. longifolia</i>	1, 2	29	
<i>Aconitum romanicum</i>	1, 2	31	only traces
<i>Adonis aestivalis</i> (red)	1, 3	32	other keto carotenoids as in <i>A. annua</i>
<i>A. aestivalis</i> var. <i>citrinus</i> (yellow)	1, 2, 4, 17, 19, 22	33	no keto carotenoids
<i>Adonis annua</i>	3	34–36	also 3-hydroxy-4-keto-4,4'-dihydroxy-, and 3,3'-dihydroxy-4-keto- $\beta$ -carotene: no usual carotenoids present
<i>Aesculus hippocastanum</i>	1, 2, 4, 17, 21, 22	37	also capsanthin (1.30) and capsorubin (1.59)
<i>Aloe vera</i>	6	38	
<i>Arnica montana</i>	2, 4, 5,	39, 40	
<i>Aquilegia transsilvanica</i>	1, 2	31	only traces
<i>Astragalus</i> spp.	1, 2, 4, 7, 12, 14, 17, 19, 21, 22	32	18 species examined
<i>Bignonia</i> sp.	8	41	
<i>Calendula arvensis</i>	1, 2, 5, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20	42	
<i>Calendula officinalis</i>	1, 2, 4, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22	42–46	Lycopene absent from yellow varieties
<i>Caltha palustris</i>	1, 2, 5, 19, 21	47–50	trollixanthin = neoxanthin (1.47) $\alpha$ -carotene > $\beta$ -carotene; calthaxanthin may be a stereoisomer of lutein
<i>Campanula transsilvanica</i>	1, 2, 5	31	only traces
<i>Canna indica</i>	17	8	<i>cis</i> -isomer of 17
<i>Canna</i> spp.	9	41	
<i>Centaurea ratesatensis</i>	1, 2	31	only traces
<i>Cheiranthus semmoneri</i>	2	34	
<i>Chrysanthemum cinerariaefolium</i>	2	19	mainly esters and some <i>cis</i> -isomers

<i>Species</i>	<i>Pigments</i>	<i>Ref.</i>	<i>Notes</i>
<i>Chrysanthemum coronarium</i>	15, 16, 24	51	
<i>Chrysanthemum</i> spp.	1, 2, 5, 16	52, 53	
<i>Citrus limonium</i>	1, 2, 5, 17, 19, 25	54	Also <i>cis</i> -violaxanthin
<i>Crepis aurea</i>	1, 2, 17, 21	40	
<i>Crocus sativus</i>	1, 4, 13, 14, 21	55, 56	also crocetin (1.27) and related compounds
<i>Cucurbita pepo</i>	1, 2, 4, 5, 17, 19, 22, 25, 26	57–59	Petaloxanthin is a mixture of zeaxanthin and lutein with traces of antheraxanthin
<i>Cytisus laburnum</i>	17	47	
<i>C. scoparius</i>	1, 2, 5, 12, 16, 19, 21	49, 60	trollixanthin = neoxanthin (1.47)
<i>Delonia regia</i>	1, 2, 4, 5, 7, 9, 10, 11, 12, 13, 15, 16, 17, 18, 19, 22, 24, 25, 27	61	
<i>Delphinium simonkoianum</i>	1, 2	31	only traces
<i>Dianthus callizonus</i>	1, 2	31	only traces
<i>Dimorphotheca aurantiaca</i>	1, 7(?), 8, 9, 10, 12, 14, 19, 21, 24, 27	49, 62	also $\beta$ -zeacarotene (2.12)
<i>Doronicum paradlanches</i>	2	39	
<i>Eschscholtzia californica</i>	2, 8, 17, 25	41, 63–65, 66	major pigment eschscholtzxanthin (1.25)
<i>Forsythia europaea</i>	2, 4, 5, 15, 17, 19, 22, 29	66, 67	
<i>F. intermedia</i>			
<i>F. suspensa</i>		66	
<i>F. viridissima</i>		66	
<i>F. spp.</i>	17	8	<i>cis</i> isomers
<i>Fremontia californica</i>	8	41	
<i>Gazania rigens</i>			
(a) Californian	1, 2, 8, 9, 10, 12, 14, 16, 22, 24, 27, 28	41, 68–70	also $\beta$ -zeacarotene (2.12)
(b) Portuguese	1, 2, 7, 12, 28	71	
(c) English	28	62	
<i>Gelsemium sempervivens</i>	8	41	
<i>Genista oligosperma</i>	1, 2, 5, 16, 17, 21	31	eloxanthin = lutein 5,6-epoxide
<i>G. tridentata</i>	1, 2, 21	72	
<i>Gentiana phogifolia</i>	1, 2	31	only traces
<i>Gerbera jamesonii</i>	1, 8, 16, 18, 22, 24, 27	62	yellow-orange and red-orange strains contain the same carotenoids
<i>Geum</i> spp.	1, 2, 4, 5, 8, 10, 15, 16, 18, 19, 20, 21, 22, 24, 25, 30, 33, 34	73	

Species	Pigments	Ref.	Notes
<i>Grevillea robusta</i>	1, 2, 22	74	
<i>Helenium autumnale</i>	2	30, 39	
<i>Helianthus annuus</i>	1, 2, 5, 7, 17, 22	8, 30, 31, 75-77	Taraxanthin (= mixture of lutein 5,6-epoxide, flav-oxanthin and chrysanthemaxanthin); also <i>cis</i> isomers of 17
<i>Hepatica transsilvanica</i>	1, 2, 5	3	only traces
<i>Heracleum palmatum</i>	1, 2, 17	31	only traces
<i>Hibiscus syriacus</i>		78	
<i>Hieracium aurantiacum</i>	1, 5, 8, 15, 16, 18, 22, 24, 27	62	
<i>H. pilosella</i>	1, 2, 5, 15, 16, 18, 22, 24, 27	62	
<i>Heliopsis scabrae major</i>	2	30	
<i>Hypericum perforatum</i>	2, 17, 29	79	trollixanthin = neoxanthin (1.47)
<i>Hypochoeris radicata</i>	1, 2, 8, 10, 15, 16, 18, 21, 24	62	also $\beta$ -zeacarotene (2.12)
<i>Impatiens noli me tangere</i>	2, 4, 5, 15, 17, 19, 22, 29	8, 67, 77, 80	also <i>cis</i> -isomer of 17
<i>Iris pseudacorus</i>	1, 2, 19	81	
<i>Jacaranda ovalifolia</i>	1, 8	41	phytofluene (1.37) > $\beta$ -carotene (1.13)
<i>Kerria japonica</i>	1, 2, 5	82, 83	
<i>Laburnum anagyroides</i>	1, 2, 5, 19	47, 82	
<i>Lathyrus transsilvanicus</i>	2, 20	80	
<i>Leontodon autumnalis</i>	2, 20	80	
<i>Lilium candidum</i>	17, 25(?)	84	
<i>L. spp. (yellow)</i>	1, 4, 22	85	
<i>L. spp. (orange)</i>	36, 37, 38	85	
<i>Linum flavum</i>	17	8	<i>cis</i> -isomers of 17
<i>Linum uninode</i>	1, 2, 4, 16, 17	31	
<i>Lotus corniculatus</i>	1, 2, 5, 17, 21	40	
<i>Lysimachia punctata</i>	17	8	<i>cis</i> -isomers of 17
<i>Marattia praecox</i>	1, 21	86	$\alpha$ - > $\beta$ -carotene
<i>Medicago falcata</i>	2, 5, 17	87, 88	
<i>M. sativa</i>	}	89	mainly xanthophylls
<i>M. platycarpas</i>			
<i>Mimulus cupreus</i>			
<i>M. guttatus</i>	de-epoxyneoxanthin, main pigment	91-93	also mimulaxanthin (5.2)
<i>M. longiflorus</i>	1, 4, 8, 12, 13, 14, 22, 30	41, 94, 95	also <i>pro</i> - $\gamma$ -carotene
<i>M. tigrinus</i>	1, 20, 22	90	
<i>Narcissus poeticus</i>	1, 2, 4, 5, 7, 8, 9, 10, 15, 16, 18, 21	9	also $\beta$ -zeacarotene (2.12)
<i>N. pseudonarcissus</i>	1, 2, 5, 9, 16, 18, 22, 24, 27	9, 96	also <i>cis</i> -isomers of $\beta$ -carotene-5,6,5',6'-diepoxide

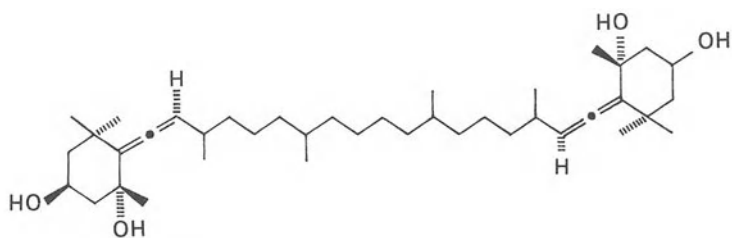
<i>Species</i>	<i>Pigments</i>	<i>Ref.</i>	<i>Notes</i>
<i>N. spp.</i>	2	39	
<i>Oenothera spp.</i>	1, 2, 17, 19(21), 22	97, 98	
<i>Osmanthus fragranse</i>	1, 21	99, 100	
<i>Photinia spp.</i>	8	41	
<i>Phyteuma vagneri</i>	1, 2	31	
<i>Potentilla erecta</i>	1, 2	101	
<i>P. gusuleacii</i>	1, 2, 5, 17	31	
<i>P. repens</i>	1, 2, 5, 15, 16, 19, 22, 27, 29, 33	73	an $\alpha$ -carotene diepoxide (which is not a known structure) is also reported.
<i>P. cultivar (Woodbridge gold)</i>	1, 2, 5, 8, 15, 16, 19, 24, 25, 27, 31	73	
<i>Pyracantha coccinea</i>	1, 14, 22	57	
<i>Ranunculus acer</i>	1, 2, 5, 16, 17, 20, 21, 24, 32, 39	77, 102– 104	
<i>R. arvense</i>	1, 2	82	
<i>R. flabellifolius</i>	1, 2, 5, 17, 20, 25	31	
<i>R. steveni</i>	2	105	
<i>Rhododendron kotskyi</i>	1, 2, 5	31	
<i>Rosa foetida persiana</i>	1, 2, 14, 17, 18, 23, 40, 41	105A	
<i>Rosa spp.</i>	1, 2, 5, 7, 8, 10, 12, 14, 16, 18, 19, 21, 22, 23, 24, 25, 27, 29, 30, 33, 34	73	rosaxanthin (unknown structure)
<i>Rudbeckia neumanii</i>	2	39	
<i>Senecio doronicum</i>	4	82	
<i>S. scandens</i>	1, 2, 4, 8, 10, 15	62	also $\beta$ -zeacarotene (2.12)
<i>Silene dubia</i>	1, 2	31	
<i>Silphium perfoliatum</i>	2	55	
<i>Sinapsis officinalis</i>	1, 17	82	
<i>Sorbus dacica</i>	1, 2	31	
<i>Spartium junceum</i>	8	41	
<i>Tagetes aurea</i>	2	82	
<i>T. erecta</i>	1, 2, 4, 5, 8, 9, 15, 16, 17, 18, 21, 22, 24, 25, 27, 31	39, 62, 106	also $\beta$ -zeacarotene (2.12)
<i>T. grandiflora</i>	1, 2, 12, 17, 20	39	
<i>T. nana</i>	2	39	
<i>T. patula</i>	1, 2, 5, 7, 9, 10, 14, 15, 16, 17, 18, 21, 22, 27, 32	39, 62, 103, 107	also $\beta$ -zeacarotene (2.12)
<i>Taraxacum officinale</i>	2, 4, 5, 7, 16, 17, 19, 22, 25, 27	67,77, 108– 111	taraxanthin = lutein, 5,6-epoxide
<i>Tecoma capensis</i>	1, 4, 7(?), 8, 12, 14	41, 112	

<i>Species</i>	<i>Pigments</i>	<i>Ref.</i>	<i>Notes</i>
<i>T. stans</i>	1, 2, 4, 8, 9, 10, 15, 17, 22, 25, 35	112, 113	
<i>Torenia bailloni</i> }	2, 4, 17, 19, 25,	114,	
<i>T. fourmieri</i> }	35(?)	115	
<i>Tragopogon pratensis</i>	1, 2, 5, 16, 17, 21, 30	82, 103	
<i>Trollius europaeus</i>	1, 2, 5, 23	82,83	
<i>Tropaeolum majus</i>	1, 2, 4, 5, 8, 16, 18, 19, 21, 22, 28, 30, 31	13,39, 116	Trollixanthin = Trolliflor = Neoxanthin (1.47)
<i>Tulipa</i> spp. (yellow)	17	117, 118	
(golden)	1, 2, 5, 8, 9, 10, 15, 16, 18, 24, 27	62	
<i>Tussilago farfara</i>	2, 4, 5, 17, 19, 22, 25, 29	67, 110	
<i>Ulex europaeus</i>	1, 17, 21	68	
<i>U. gallii</i>	1, 16, 17, 21	68	
<i>Viola</i> (blue)	14	39	
<i>V. declinata</i>	1, 2	31	
<i>V. tricolor</i>	1, 2, 3, 14, 16, 17, 18	111, 119, 120	only deep coloured varieties contain 14
<i>V. yooi</i>	1, 2	31	
<i>Zinnia elegans</i>	8	41	

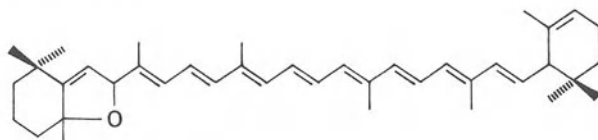
## Key

- |   |   |
|---|---|
| 1. $\beta$ -Carotene (1.13)                           | 21. $\alpha$ -Carotene (1.12)   |
| 2. Lutein (1.74)                                      | 22. $\beta$ -Cryptoxanthin (1.60)                                     |
| 3. Astaxanthin (1.68)                                 | 23. Mutatoxanthin (5.4)   |
| 4. Zeaxanthin (1.18)                                  | 24. $\beta$ -Carotene 5,6-epoxide                                     |
| 5. Lutein 5,6-epoxide                                 | 25. Antheraxanthin (1.24)   |
| 6. Rhodoxanthin (4.8)                                 | 26. Luteoxanthin (5.5)  |
| 7. Rubixanthin (4.9)                                  | 27. $\beta$ -Carotene 5,6,5',6' diepoxide                             |
| 8. Phytofluene (1.37)                                 |   |
| 9. Phytoene (1.36)                                    | 28. Gazanixanthin (1.56)  |
| 10. $\zeta$ -Carotene (1.14)                          | 29. $\beta$ -Cryptoxanthin 5,6-epoxide                                |
| 11. $\delta$ -Carotene (4.4)                          | 30. $\alpha$ -Carotene 5,6-epoxide                                    |
| 12. $\gamma$ -Carotene (1.38)                         | 31. $\alpha$ -Cryptoxanthin (4.1)                                     |
| 13. Polycopene (1.55)                                 | 32. Rubichrome (5.6)  |
| 14. Lycopene (1.11)                                   | 33. Zeaxanthin 5,6-epoxide  |
| 15. Mutatochrome (1.44)                               | 34. Aurochrome (5.7)  |
| 16. Chrysanthemaxanthin (4.2),<br>Flavoxanthin* (4.3) | 35. $\beta$ -Cryptoxanthin 5,6,5',6'-diepoxide                        |
| 17. Violaxanthin (1.58)                               | 36. Echinonone-like   |
| 18. Auroxanthin (1.62)                                | 37. Capsanthin (1.30)   |
| 19. Neoxanthin (1.57)                                 | 38. Capsorubin (1.59)   |
| 20. Flavochrome (5.3)                                 | 39. 5,6-Dihydro- $\beta,\epsilon$ -carotene 3,5,6,3'-<br>tetrol (5.8) |

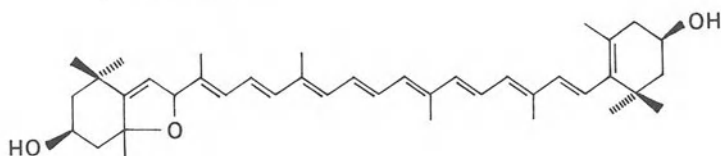
\*Spatial isomers



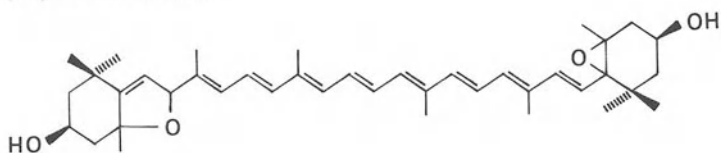
(5.2) Mimulaxanthin



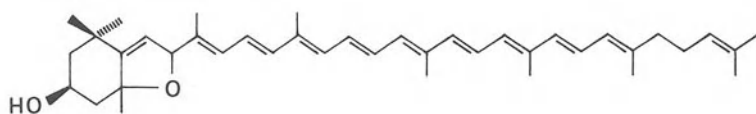
(5.3) Flavochrome



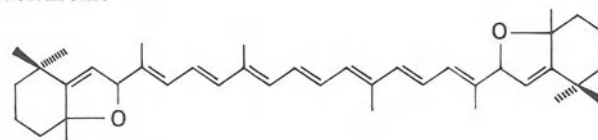
(5.4) Mutatoxanthin



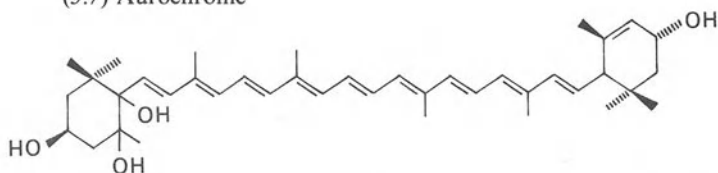
(5.5) Luteoxanthin



(5.6) Rubichrome



(5.7) Aurochrome



(5.8) 5,6-Dihydro- $\beta,\epsilon$ -carotene-3,5,6,3'-tetrol

The xanthophylls in flower petals are usually esterified and helenein (lutein dipalmitate) was first isolated in 1930 from *Helianthus annuus* [30, 39]. More recent investigations have shown that in this source the other xanthophylls present are also esterified [115] and that stearic (C<sub>18</sub>), myristic (C<sub>14</sub>) and lauric (C<sub>12</sub>) acids are involved as well as palmitic acid; acetates were also observed [76]. A similar pattern exists in petals of *Forsythia intermedia*, *Taraxacum officinale*, *Tussilago farfara* and *Impatiens noli-me tangere* with myristates predominating [67]. In *Taraxacum officinale* lutein 5,6-epoxide (taraxanthin) exists in both mono- and diesterified forms [121].

The diapocarotenoic acid crocetin (1.27) occurs naturally in petals of saffron (*Crocus sativus*) as the digentiobiosyl ester crocin and, to a limited extent, free [122]. A number of sugar esters, possibly intermediates in crocetin formation, have recently been identified [56, 123, 124]. These include the  $\beta$ -D-gentiobiosyl- $\beta$ -D-glucosyl ester, the di- $\beta$ -D-glucosyl ester and the mono- $\beta$ -D-glucosyl and  $\beta$ -D-gentiobiosyl esters, as well as the diester in which crocetin contains one molecule of  $\beta$ -D-glucose and one of  $\beta$ -D-gentiobiose. Crocin is also found in petals of *C. albifloris* [125], *C. luteus* [126], *Cedrela toona* [126], *Nyctanthes arbor-tristis* [125, 126] and *Verbascum phlomoides* [127]. It is interesting that the corresponding dialdehyde and monoaldehyde of crocetin have been obtained from leaves of *Jacquinia angustifolia* [128].

#### (b) Quantitative distribution

The levels of carotenoids in flower petals vary considerably from 20–45 mg/100 g fresh weight in *Tecoma* [112, 129] and *Delonia* [61], to 2% dry weight in the red coronas of cultivated narcissi, whilst an astounding value of 16.5% dry weight is reached in the deep red fronds of the corona of *Narcissus poeticus*

Table 5.5 Distribution of carotenoids in petals of *Tecoma stans* [113]  
(Total pigment concentration 45 mg/100 g fresh wt.)

Carotenoids	% of total polyenes
Phytoene (1.36)	0.15
Phytofluene (1.37)	0.29
$\beta$ -Carotene (1.13)	1.10
$\xi$ -Carotene (1.14)	0.11
Mutatochrome (1.44)	0.11
$\beta$ -Cryptoxanthin (1.60)	10.25
$\beta$ -Cryptoxanthin-5,6-epoxide	1.05
$\beta$ -Cryptoxanthin 5,6,5',6' diepoxide	0.24
Zeaxanthin (1.18)	3.11
Lutein (1.74)	4.15
Violaxanthin (cis) (1.58)	47.27
Antheraxanthin (1.24)	32.11
Unknowns	0.09



*recurvis* [96]. A typical quantitative distribution is illustrated in Table 5.5 for *Tecoma stans*.

### (c) Localization

Carotenoids in flower petals are localized in chromoplasts which in many cases develop from chloroplasts and have irregular amoeboidal shape [130]. The structure of a chromoplast from the trumpet of a daffodil (Golden Harvest) is illustrated in Fig. 5.1 [131]. The mature organelle is composed solely of numerous concentric membranes [132]. A similar situation exists in the petals of *Tulipa* [133]. The  $\beta$ -carotene in the chromoplasts of the corona of *Narcissus poetica* occurs as crystals which have been characterized crystallographically [129, 134] and which are localized in the intra-thylakoidal space [129]. The situation is different in *Ranunculus repens* in which the yellow chromoplasts are homogeneous osmiophilic globuli, up to 150 nm in diameter, which first appear in the developing chloroplast or leucoplast. The globuli form between the lamellae and eventually by increasing their size and number they destroy the lamellar structure [135, 136]. A similar structure is observed in the flowers of chrysanthemums (*C. segetum*) [137], *Davidia* [138], tulips [139], *Aloe plicatilis* [140] and *Spartium junceum* [137]. The carotenoids in cauliflower florets are particle-bound [141].

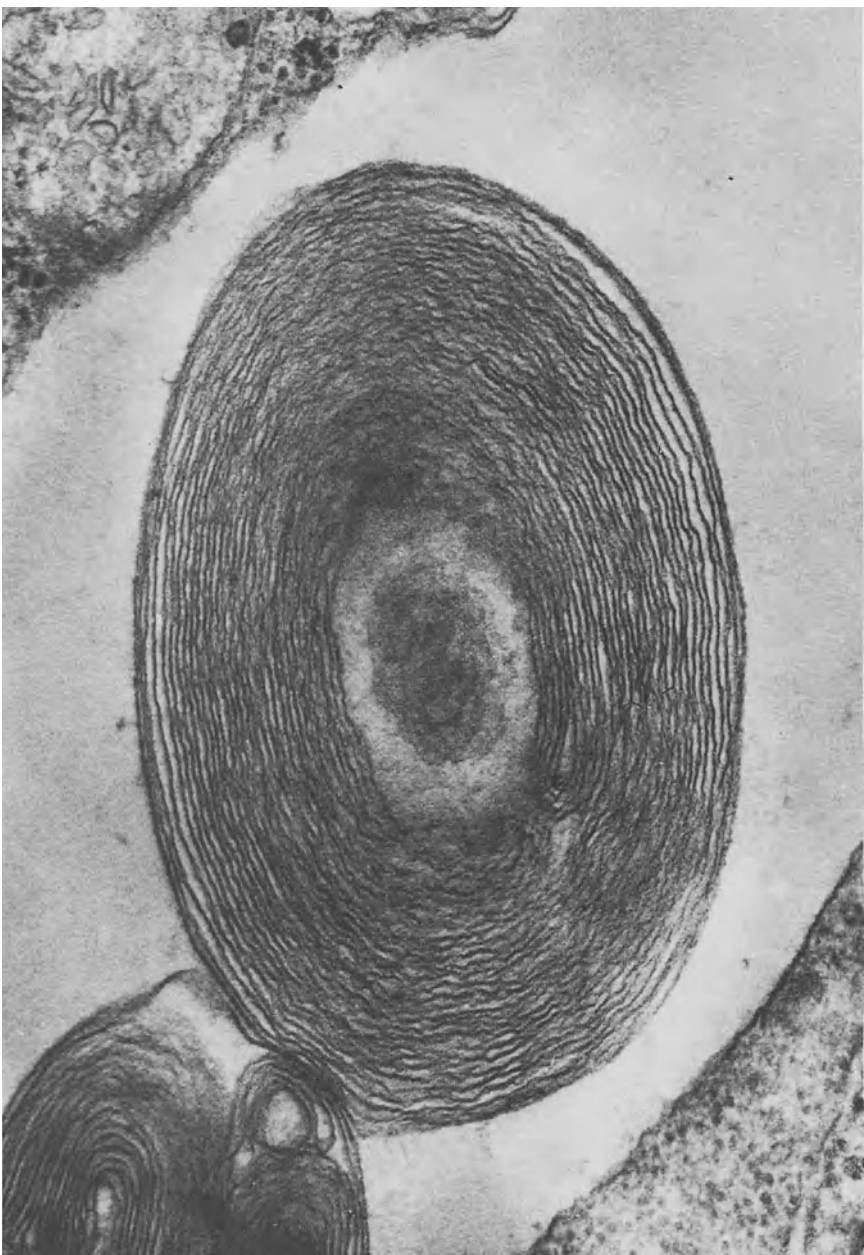
### (d) Metabolism

Studies on orange *Calendula officinalis* show that the total carotenoid level increases some eight times during flower development, the greatest increase being in  $\gamma$ -carotene (1.38) and lycopene (1.11) and the least in  $\beta$ -carotene (1.13) [95]. Flowers of *Viola tricolor* collected in the spring contain less auroxanthin (1.62) and violaxanthin (1.58) than do autumn flowers [142]. The petals of flowers from plants grown at high altitudes contain more xanthophylls and less carotenes than those from plants grown at normal altitudes [143]. When petals of *Mimulus longiflorus* develop on the plant they contain no *cis*-carotenoids, but if excised buds are kept in diffuse light considerable amounts of poly-*cis* lycopene and pro- $\gamma$ -carotene are formed when the blossoms open [95].

In the bright red edges of the corona of *Narcissus* spp.  $\beta$ -carotene synthesis can reach the rate of 70  $\mu\text{g}/\text{mg}$  dry wt./day, which is over  $10^4$  times faster than in carrot roots [96]. In chrysanthemums, however, carotenoids increased to a maximum in half opened flowers and then decreased [144]. Antholysis of flowers of *Sesamum indicum* causes an increase in the amount of carotenoids present [145].

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Fig. 5.1. Electron micrograph of a chromoplast from the petals of a daffodil ( $\times 80\,000$ ) (kindly provided by Dr A. T. James and Dr J. M. Stubbs).



*Fig. 5.1. Electron micrograph of a chloroplast from the petals of a daffodil ( $\times 80\,000$ ) (kindly provided by Dr A. T. James and Dr J. M. Stubbs).*

(e) *Contribution to flower colour*

Many lemon and yellow coloured flowers contain large amounts of xanthophylls, particularly epoxides [146, 147] whilst the deeper orange petals are often characterized by increased amounts of  $\beta$ -carotene as in the orange fringes of *Narcissus* spp. [69] and the Red Emperor variety of *Mimulus cupreus* [90], or lycopene as in the very deep orange varieties of *Calendula officinalis* [45], *Viola tricolor* [30] and *Tagetes erecta* [62]. In yellow lilies (e.g. *Lilium hansonii*),  $\beta$ -carotene (1.13),  $\beta$ -cryptoxanthin (1.60) and zeaxanthin (1.18) predominate whilst in some orange lilies (*L. pumilum*) capsanthin (1.30) and capsorubin (1.59) are the major components. The orange *L. dauricum* synthesizes an echinenone-like pigment [85]. Yellow roses owe their colour to the presence of carotenoids, mainly epoxides [73, 105A]; blends such as 'Masquerade' accumulate both carotenoids and anthocyanins [111A].

(f) *Taxonomic studies*

Carotenoid patterns have been helpful in determining taxonomic relations in *Linum* spp. For example all members of the *L. schiedeanum*, *L. virginianum*, *L. neomexicanum* and *L. zulcatum* complexes contain violaxanthin (1.58) whilst all those of the *L. rigidum* complex with the exception of *L. subertes* contain lutein (1.74), and its 5,6-epoxide. *L. subertes* in synthesizing violaxanthin retains a chemical link with the other complexes. These observations support conclusions from early morphological, chromosomal and pollen studies [148].

(g) *Mutant studies*

The presence or absence of a carotenoid pigment in flowers of *Cheiranthus cheirii* is governed by a gene T [149]; the pigment is absent from plants homozygous for the recessive allele. There are genes in *Eschscholtzia californica* which determine the amount of carotenoid formed, which carotenoids are formed and which areas of the petal contain the pigment [150]. A sterile hybrid of *Torenia fournieri* and *T. ballonia* is very low in carotenoids [151].

(h) *Tissue cultures*

Florets of *Chrysanthemum monifolium* cultured at 15°C with a 12 h day length produced considerable amounts of carotenoids [152].

## 5.1.3 SEEDS

(a) *Cereals – general*

In mature cereal seeds used in human and avian nutrition the carotenoid level is

about 100  $\mu\text{g}/100\text{ g}$  fresh weight of which about 10% is carotene [153–156]. In the case of wheat this represents 14.5% of the unsaponifiable matter [157].  $\beta$ -Carotene (1.13) is usually the only carotene present but  $\alpha$ -carotene (1.12) is said to be the major pigment in millet [158] and lycopene (1.11) has been isolated from chaura (*Maytenus disticha*) seeds [159]. The amount of  $\beta$ -carotene as a percentage of total pigment present varies from 'a trace' to 26% in varieties of Sorghum with yellow endosperms [160], levels falling within the range 0.084–1.18 p.p.m. [161].

The xanthophyll mixture in cereal seeds is complex and generally similar to that in green leaves with lutein the major component [162–166].

### (b) *Maize*

The pigments of maize have been studied extensively over a long period; indeed zeaxanthin (1.18) and  $\beta$ -cryptoxanthin (1.60) were first isolated from this source [167]. Other carotenoids present include  $\beta$ -carotene (1.13) [167],  $\alpha$ -carotene (1.12) [168],  $\beta$ -zeacarotene (2.12) [169],  $\zeta$ -carotene (1.14) [168],  $\gamma$ -carotene (1.38) [168],  $\alpha$ -cryptoxanthin (zeinoxanthin) (4.1) [170] and lutein (1.74) [171].

Numerous reports exist on the qualitative and quantitative variations in the carotenoids of maize varieties and hybrids grown in many countries [167, 168, 172–179]. Table 5.6 indicates the maximum quantitative variations observed in eleven pigments in inbred strains from the US Midwest; 125 strains were examined [177].

A caroteno-protein complex containing  $\beta$ -apo-8'-carotenoic acid has been isolated from maize [180]. A genetic analysis of maize has stimulated an unexpected conclusion that there are two separate biosynthetic pathways for carotenes and xanthophylls [181] (see Chapter 2).

### (c) *Legumes*

Green soya beans (*Soja glycine*) [182], cow peas (*Vigna sinensis*) [182], Lima beans (*Phaseolus lunatus*) [183], peas (var. Thomas Laxton) [184, 185] and French (snap) beans (*P. vulgaris*) [186, 187] contain between 2 and 7  $\mu\text{g}/\text{g}$  (fresh wt.) of carotene. A survey showed concentrations varying from 3.8–5.6  $\mu\text{g}/\text{g}$  (wet wt.) for early peas and 3.38–7.14 for late peas [188] but such marked changes were not always observed [189]. US cultivars of Lima beans contain 0.017–0.024  $\mu\text{g}/\text{g}$  (dry wt.) whilst those of cultivars from outside the US have higher levels, for example one from Colombia contains 1.86  $\mu\text{g}/\text{g}$  (dry wt.) [190].

The qualitative distribution in peas and beans is probably very similar to that in leaves [182, 183]. In developing pea seeds the accumulation of carotenoids parallels the synthesis of starch until just before maturation when the pigment levels begin to drop rapidly [191–193]. In French beans the

Table 5.6 Maximum and minimum values for 11 carotenoids in 125 inbred maize strains from US midwest\* [177]

Strain	Phytoene (1.36)	Phyto- fluene (1.37)	$\beta$ -Carot- ene (1.13)	$\beta$ -Zea- carotene (2.12)	$\zeta$ -Carot- ene (1.14)	$\alpha$ -Crypt- oxanthin (4.1)	$\beta$ -Crypt- oxanthin (1.60)	Zeaxan- thin (1.18) (esters)	Lutein (1.74)	Zeaxan- thin (1.18)	Poly- hydroxy pigments
Oh45	25.6	9.3	4.4	4.0	4.2	2.2	3.2	4.0	29.2	5.4	1.3
Oh26A	29.2	7.2	3.1	<b>4.7</b>	1.0	2.0	5.1	0.8	17.8	17.6	1.7
Mo <sup>3</sup> = Mo0221	17.4	4.1	<b>5.0</b>	1.8	1.7	0.4	1.4	<b>4.1</b>	6.8	3.1	0.5
A25	24.0	6.2	4.0	4.6	1.1	<b>7.8</b>	1.1	0.8	23.0	4.1	1.1
H54	14.0	4.5	4.7	0.7	1.4	0.6	2.1	<b>0.3</b>	13.3	1.2	1.8
Kys	19.5	5.6	2.4	2.4	1.4	0.8	<b>5.2</b>	1.4	10.1	<b>27.4</b>	<b>3.9</b>
Ky126	16.7	3.9	2.5	1.2	1.6	2.6	2.6	0.6	25.3	9.9	2.9
A430	14.0	3.0	3.3	0.4	0.5	2.9	1.5	0.7	11.7	2.7	<b>0.4</b>
K770	24.4	6.3	2.1	1.7	1.6	3.6	2.5	1.0	<b>33.1</b>	10.5	1.2
T92	<b>45.8</b>	<b>10.2</b>	1.1	2.4	1.8	1.0	2.0	1.6	18.2	7.3	1.4
C131A	12.8	1.8	1.2	0.7	0.5	0.8	1.5	0.3	13.0	<b>0.6</b>	1.3
Mo940	14.6	2.2	1.4	<b>0.0</b>	<b>0.0</b>	1.2	1.4	0.4	14.0	3.0	2.6
H14	10.9	1.5	1.1	0.2	0.3	<b>0.1</b>	1.4	1.0	4.2	5.9	3.2
J557	12.2	2.4	0.5	1.2	0.8	0.0	1.1	1.7	<b>2.0</b>	6.6	1.6
H58	<b>5.6</b>	<b>0.8</b>	0.5	0.1	0.2	0.2	0.8	0.4	8.3	5.4	1.8
Pa86	11.9	1.5	<b>0.1</b>	0.1	0.1	0.1	<b>0.3</b>	0.4	2.4	1.8	0.4

\* Maximum and minimum values for each pigment are indicated in bold type.

variation in carotenoid concentration in the beans during maturation does not parallel that in the pod [186]. Early on the levels in the seeds are much higher than in the pods but they drop considerably at maturity, whilst the pod levels do not alter significantly until they become markedly over-mature. The level of  $\beta$ -carotene also drops markedly on maturation [194]. Peas which produce small seeds have higher carotene levels than normal [195].

(d) *Miscellaneous*

The carotenoid distribution in some other seeds is summarized in Table 5.7. Two points need emphasis: (i) the seeds of *Brachychilum* contain lycopene

Table 5.7 Carotenoids in some miscellaneous seeds

Seed	Pigments	Ref.
<i>Aglaonena commutatum</i>	Lutein	196
<i>Argania spinosa</i>	Xanthophylls but no carotenes	197
<i>Bixa orellana</i>	Bixin (5.9); no conventional carotenoids	198, 199
<i>Brachychilum horsfieldi</i>	Lycopene (1.11)	200
<i>Capsicum annuum</i>	Capsanthin (1.30); capsorubin (1.59)	201
<i>Carya</i> sp. (pecan)	Leaf xanthophylls	202
<i>Cucurbita</i> sp. (pumpkin)	Leaf xanthophylls	203
<i>Cycas revoluta</i>	$\beta$ -Carotene (1.13); $\beta$ -cryptoxanthin (1.60); zeaxanthin (1.18) (major)	204
<i>Euonymus europaeus</i>	Zeaxanthin (1.18)	205
<i>Haloxylon</i> sp.	Leaf xanthophylls	206, 207
<i>Lycopersicon</i> sp.	$\beta$ -Carotene (1.13) trace; no lycopene (1.11)	208
<i>Momordica charantia</i>	Lycopene (1.11) with traces of phytofluene (1.37); $\beta$ -carotene (1.13); $\zeta$ -carotene (1.14); $\alpha$ -cryptoxanthin (4.1); lutein (1.74); $\beta$ -cryptoxanthin (1.60) and rubixanthin (4.9)	208
<i>Ternstroemia japonica</i>	Ternstroemiaxanthin (5.10)	209
<i>Zamia</i> sp.	Mono and dihydroxy- $\beta$ -carotenes	210

(1.11) whilst the pericarp accumulates  $\beta$ -cryptoxanthin (1.60) [200]; (ii) the seeds of *Momordica charantia* contain twelve times as much lycopene than does the pericarp and become bright red on ripening of the fruit because of the very large amounts of lycopene (2.7 mg/g) synthesized at this time [208].

(e) *Factors controlling synthesis*

Addition of fertilizers generally improves the carotenoid content of maize [211, 212] although high supplements of urea reduce the carotenoid content not only of maize but also of millet (*Pennisetum glaucum*) [213]. Molybdenum

seems particularly effective in improving carotene levels in maize [214], peas [215] and lupin [216]; cobalt has a similar effect in maize [214]. Apart from the effect of trace elements it is generally considered that carotenoid levels in maize are reasonably stable to pre-harvest influence [217]. Apparently one of the better means of improving carotenoid levels in maize is to reduce the plant density [218].

The application of the soil fumigants Telone (1,3-dichloropropene) and Nemagon (1,2-dibromo-3-chloropropane) increased carotenoid levels in sweet corn [219].

#### 5.1.4 FRUIT

##### (a) *Qualitative distribution*

During the past forty years there have been numerous investigations into the nature of fruit carotenoids. The inevitable advances in our knowledge of carotenoid chemistry and improvements in analytical techniques have rendered some of the earlier investigations incomplete rather than incorrect, but some mis-identifications have occurred. The known distribution in fruit is given in Table 5.8 and some general patterns of distribution emerge although inevitably patterns tend frequently to merge into each other [220]. Eight main groups can be detected in which one finds in the fruit: (i) insignificant amounts of carotenoids: (ii) small amounts generally of the pigments found in chloroplasts: (iii) relatively large amounts of lycopene (1.11) and the related partly saturated acyclic polyenes, phytoene (1.36), phytofluene (1.37),  $\zeta$ -carotene (1.14) and neurosporene (2.14): (iv) relatively large amounts of  $\beta$ -carotene (1.13) and its oxidized derivatives  $\beta$ -cryptoxanthin (1.60) and zeaxanthin (1.18): (v) comparatively large amounts of epoxides, particularly furanoid epoxides: (vi) unique carotenoids such as capsanthin (1.30): (vii) poly-*cis* carotenoids such as prolycopene (1.55): (viii) apocarotenoids such as  $\beta$ -citraurin (1.26).

The xanthophylls in fruit are, as in petals, almost entirely esterified. For example, physalein, first isolated from *Physalis alkekengi*, is zeaxanthin dipalmitate [221] and caricaxanthin from *Carica papaya* [222] and *Citrus pooenis*, is  $\beta$ -cryptoxanthin palmitate [223]. A recent investigation of tomato xanthophylls has revealed the presence for the first time in nature of a series of acyclic 1,2-epoxides derived from phytoene (1.36), phytofluene (1.37),  $\zeta$ -carotene (1.14) and lycopene (1.11) [224, 225] (Table 5.8). Phytofluene epoxide may be identical with the polyene previously described as phytofluenuol [226]. These polyenes have also been observed in the berries of *Solanum dulcamara* [227] so they may eventually be distributed widely in trace amounts. The apocarotenoids apo-6'-lycopenal (5.14) and apo-8'-lycopenal are also present in tomatoes [225, 228]. They probably represent degradation products of lycopene.

Table 5.8 Carotenoid distribution in fruit

Source	Pigments	Major pigment patterns (see text)	Ref.	Notes
<i>Actinophloeus angustifolia</i>	1	3	229	only 1 reported
<i>A. macarthurii</i>	1	3		
<i>Aglaeonema commutatum</i>	1, 2, 3, 4, 7, 11, 17, 18	3	197	compare seeds (Table 5.7)
<i>A. nitidum</i>	1	3		
<i>A. oblongifolium</i>	1	3	229	only 1 reported
<i>A. simplex</i>	1	3		
<i>Ananas sativus</i>	2, 3	22	230	
<i>Arbutus unedo</i>	1, 2, 3, 4, 5, 6, 7	3, 4	231	
<i>Archontophoenix alexandrae</i>	1	3	229	only 1 reported
<i>Area alicae</i>	1	3	229	only 1 reported
<i>Aronia melanocarpa</i>	1	?	232	
<i>Arum italicum</i>	1	3	229, 233	
<i>A. maculatum</i>	1, 2, 8, 9, 10, 11, 19	3, 7	236	poly <i>cis</i> lycopenes present
<i>A. orientale</i>	1, 2, 3	3, 7		
<i>Asparagus officinale</i>	5	4	234	only 5 reported
<i>Atropa belladonna</i>	2, 5	4	235	
<i>Attalea gomphococca</i>	2	4	237	only 2 reported
<i>Berberis barbarossa</i>	12		235	capsanthin present in most
<i>B. vulgaris</i>	12	2, 4, 6	237, 239	species
<i>B. spp. (34)</i>	2, 3, 4, 6, 13, 14, 15			
<i>Bixa orellana</i>	16	8	199	unique apo-carotenoid
<i>Brachychilum horsfieldi</i>	1, 5	6	201	1 in seeds, 5 in pericarp (see also Table 5.7)
<i>Bryonia dioica</i>	1	3	229, 240	only 1 reported
<i>Butia capitata</i>	19	7	241	poly <i>cis</i> carotenes present
<i>Caliptocalix spicatus</i>	19	7	229	only 1 reported
<i>Capsicum annuum</i>	2, (?) 6, 7, 8, 9,	4, 6	235,	
var.	11, 12, 13, 14, 15,		241–	trolliflor =
<i>lycopersiciforme</i>	20, 21, 24, 33		247	neoxanthin [116]
<i>rubrum</i>				
<i>C. annuum</i> var.	2, 3, 4, 5, 7, 13,	4	243	
<i>lycopersiciforme</i>	22, 23, 24, 25			
<i>flavum</i>				
<i>C. annuum</i> (green)	3, 6, 7, 12, 21, 22, 24, 25, 26, 27	2	248	
<i>C. frutescens</i>	12	4	249, 250	
<i>C. japonicum</i>	2	4	249	only 2 reported
<i>Carica papaya</i>	2, 5, 8, 9, 10, 13, 15, 23, 24, 26, 28, 29, 30, 31	4	222, 251 252	1 present in red mutant



<i>Source</i>	<i>Pigments</i>	<i>Major pigment patterns (see text)</i>	<i>Ref.</i>	<i>Notes</i>
<i>Celastrus scandens</i>	32	4, 6	253	
<i>Citrullus vulgaris</i> (red)	1, 2, 4, 11	3, 4	254–256	
<i>C. vulgaris</i> (yellow)	—	7	257	similar to tangerine tomatoes
<i>Citrus aurantium</i>	1, 3, 4, 5, 6, 7, 29	4, 5, 8	258–260	
<i>C. grandis</i>	1, 4	3, 4, 5	261	
<i>C. limonium</i>	—	4	244, 262	
<i>C. madurensis</i>	1, 3, 5, 7	3, 4	263	
<i>C. paradisi</i>	1, 2, 3, 4, 5, 7, 8, 10, 13, 14, 22, 26, 27, 31	3, 4	264–266	8 is major component of peel
<i>C. poeensis</i>	2, 3, 5	4	222	
<i>C. reticulata</i>	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 13, 14, 15, 19, 26, 27, 33, 34, 35, 61	4, 5, 8	265, 267	some may be artifacts; Trollixanthin = neoxanthin
<i>C. sinensis</i>	2, 5, 7, 8, 9, 10, 13, 15, 19, 23, 24, 26, 27, 29, 33, 34, 39, 54, 65, 66, 67	4, 5, 6	268, 269, 269A, 270	trollixanthin and trollichrome are not neoaxanthins but unique apocarotenoids [269A] Peel different from pulp.
<i>C. spp.</i>	5, 7, 13, 39, 46, 62		271–273	Other compounds reported may be artifacts; $\beta$ -citralin (2.21) and $\beta$ -apo-8'-carotenal are converted into reticulataxanthin (5.16) and citranaxanthin (5.17) respectively on saponification in the presence of acetone [449, 450].
<i>Convallaria majalis</i>	1, 2, 3, 4, 11	3, 4	240	
<i>Cotoneaster bullata</i>	2, 5, 29	4, 5	235	
<i>C. frigida</i>	2, 5, 6, 15, 19			
<i>C. hebeophylla</i>	2, 5, 6, 15			
<i>C. occidentalis</i>	3, 7	1	274	only traces
<i>Crataegus azarolus</i>	2, 4	4, 5	275	only carotenes reported
<i>C. oxycantha</i>	3, 5, 27, 29	4, 5	235	
<i>C. pratensis</i>	2, 5, 29	4, 5	235	
<i>Cucumis citrullus</i>	1	3	240	only 1 reported
<i>C. melo</i>	2, 3, 4, 6, 7, 8, 9, 10, 26	4	276	
<i>Cucurbita maxima</i>	2, 3, 4, 6	2	258	
<i>Cuscuta salina</i>	35	6	277	

Source	Pigments	Major pigment patterns (see text)	Ref.	Notes
<i>C. subinclusa</i>	35	6	278	
<i>Cycas revoluta</i>	2, 5, 6	4	205, 279	
<i>Cyphomandra betaceae</i>	5	4	280	5 main pigment
<i>Dioscorea cayenensis</i>	2, 7, 24, 27	2	281	xanthophylls mainly esterified
<i>Diospyros costata</i>	1, 2, 4, 5, 6, 7	3, 4	231, 282	
<i>D. kaki</i>	1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 13, 14, 20, 22, 24, 25, 26, 27, 28, 29, 33, 36, 37	3, 4	283–285	lycopenes vary from 0–40% of total
<i>Dura nigrescens</i>	} 1, 2, 4, 8, 9, 10	3, 4, 7	286	
<i>D. virescens</i>				
<i>Elaeagnus longipes</i>	—	3	287	
<i>Elaeis guineensis</i>	1, 2	3, 4	288, 289	
<i>E. melasiococca</i>	1, 2	3, 4	288	
<i>Erythroxylon coca</i>	1	3	299	} only 1 mentioned
<i>E. novogranatense</i>	1	3	290	
<i>Euonymus europaeus</i>	1, 5, 29	3, 4, 6	260, 291	
<i>E. fortunei</i>	19	7	291	
<i>E. japonicus</i>	1	3	241	only 1 reported
<i>Ficus carica</i>	2, 3, 4, 6, 7, 8, 9, 24, 26, 36, 38	2	248	
(skin)	2, 3, 7, 24		292	
<i>Fortunella</i> spp.	present	—	293	
<i>Fragaria chiloensis</i>	traces	2	248	
<i>Gardinia grandiflora</i>	24	8	55	trolloxanthin = neoxanthin
<i>Gonocaryum obovatum</i>	1, 2, 4, 11	3, 4	294	
<i>G. pyriforme</i>	1, 2, 4, 11	3, 4	294	
<i>Gossypium</i> spp.	2, 3	2	295	
<i>Guava</i> spp.	2, 4	2	296	only carotene reported
<i>Guilielma gasipaes</i>	2	2	297	only carotenes reported
<i>Hibiscus syriacus</i>	2, 4		298	seed oil
<i>Hippophae rhamnoides</i> <sup>c</sup>	1, 2, 3, 4, 5, 6, 7, 10, 11, 15, 18, 31	3	234, 235, 241, 299, 299A, 300	
<i>Iris pseudacorus</i>	7	—	301	only 7 reported
<i>Lathyrus sativus</i>	2		302	only 2 reported
<i>Lonicera japonica</i>	1, 2, 5, 6, 9, 10, 11, 27	3, 4	} 235	
<i>L. periclymenum</i>	2, 5, 6, 27	3, 4		
<i>L. rubrechtiana</i>	} 63, 64	}	303	
<i>L. webbiana</i>				

<i>Source</i>	<i>Pigments</i>	<i>Major pigment patterns (see text)</i>	<i>Ref.</i>	<i>Notes</i>
<i>Luffa</i> spp.	2, 3	2	304	
<i>Lycium barbaratum</i>	5	4	240, 305	only 5 reported
<i>L. hamimifolium</i>	5	4		
<i>Lycopersicon esculentum</i>	1, 2, 4, 8, 9, 10, 11, 17, 19, 28, 29, 30, 40, 41, 42, 56, 57, 58, 59	3, 4	Numerous (see text)	Trolliflor = neoxanthin ( <i>I.47</i> ) [116]
<i>L. hirsutum</i>	1, 2, 10, 11	3, 4 }	306	
<i>L. peruvianum</i>	1, 2, 10, 11	3, 4 }		
<i>Malpighia punicifolia</i>	2, 3, 6, 8, 9, 10, 11	2, (?3)	307	
<i>Mangifera indica</i>	2, 3, 5, 6, 7, 8, 9, 11, 13, 14, 23, 26, 27, 29	4	308, 309	Mainly 2
<i>Mormodica balsamina</i>	1, 3	3	310	
<i>M. charantia</i>	1, 2, 3, 4, 5, 6, 9, 10, 11, 15, 49, 62	3, 4	209, 310, 311	
<i>Murraya exotica</i>	43, 44	8	312	
<i>Musa cavendishi</i> (pulp)	2, 6, 7	(4?)	313	Peel similar to chloroplasts
<i>M. paradisiaca</i>	2, 3	2	314	
<i>Nenga polycephalus</i>	1	3	229	only 1 reported
<i>Palisota barteri</i>	1, 2, 5, 6, 10, 39, 46	6	315	
<i>Pandanus polycephalus</i>	1	3	229	only 1 reported
<i>Passiflora coerulea</i>	1	3	229	only 1 reported
<i>P. edulis</i>	2, 5, 9, 10, 14, 27, 46, 47	8	316, 317	
<i>Persea americana</i>	2, 3, 4, 5, 7, 10, 11, 15, 22, 24, 25, 26, 45, 54, 55	2	318, 319, 388	
<i>Physalis alkekengi</i>	2, 3, 4, 5, 6, 8, 9, 10, 22, 26, 29	4	250, 307, 320	
<i>Prunus armeniaca</i>	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 13, 14, 15, 20, 22, 26, 27, 28, 29, 33, 35, 37, 48	4	283, 321–323	trollixanthin = neoxanthin
<i>P. domestica</i>	2, 3, 4, 5, 6, 7, 8, 9, 10, 13, 14, 15, 20, 22, 24, 25, 26, 29, 33, 48	4	324, 325	trollixanthin = trolliflor = neoxanthin ( <i>I.47</i> ) [116]
<i>P. persica</i>	2, 3, 4, 5, 6, 7, 8, 9, 10, 13, 14, 19, 20, 22, 24, 26, 27, 48	3, 4	323, 326–329	trollixanthin = trolliflor = neoxanthin ( <i>I.47</i> ) [116]

Source	Pigments	Major pigment patterns (see text)	Ref.	Notes
<i>Ptychandra elegans</i>	1	3	229	only 1 reported
<i>P. glauca</i>	1	3	229	only 1 reported
<i>Punica granatum</i>	traces	2	330	
<i>Pyracantha</i>	1, 2, 19	4, 7	241, 331,	
<i>angustifolia</i>			332	
<i>P. coccinea</i>	trace	1	274	
<i>P. flava</i>	2, 3, 6, 15, 29	3	235	
<i>P. rogersiana</i>	2	1	235	
<i>P. yunnanensis</i>		4, 7	331	
<i>Pyrus</i> spp. (eating apple)	2, 3, 6, 9, 10, 15, 17, 20, 23, 24, 27, 29, 49, 50	4, 5	333, 334	major pigment varies with variety
<i>P. aucaparia</i>	2	4	335	only 2 reported
<i>P. baccata</i>	2, 3, 5, 6, 9, 10, 15, 20, 23, 27, 49, 50	4, 5	337	
<i>P. cerasus</i> (Montmorency)	2, 3, 5, 14, 20, 27, 33	4, 5	336	also 3 unknowns
<i>P. prunifolia</i>	2, 3, 6, 9, 23, 27, 50	4, 5	334	
<i>Ribes nigrum</i>	2	?	337	
<i>Rosa canina</i>	1, 2, 3, 4, 5, 8, 9, 10, 11, 15, 19, 20, 35	3, 4, 6, 8	235, 307, 338-341	
<i>R. damascena</i>	1, 2, 3, 6, 11, 35	3, 6, 8	336	
<i>R. moyesi</i>	1, 2, 3, 6, 8, 9, 10, 22, 35	3, 4, 6, 8	235	
<i>R. rubiginosa</i>	1, 2, 3, 6, 11, 20, 35	3, 4, 6	70, 340, 341	
<i>R. rubrifolia</i>	1, 2, 6, 8, 9, 15, 17, 35	3, 4, 6	235	
<i>R. rugosa</i>	1, 2, 4, 11, 35	3, 4, 5	342, 343	
<i>Rubus chamaemorus</i>	1, 2, 4, 6, 35	3, 4, 6	344	
<i>R. procerus</i>	2, 3, 4, 6, 8, 9, 10	2	330	also some epoxides
<i>Sabal serrulatum</i>	2	4	345	only 2 reported
<i>Sambucus nigra</i>	2, 3, 15, 24	2	235	
<i>Seaforita elegans</i>	1	3	346	only 1 reported
<i>Shepherdia canadensis</i>	1, 2, 8, 9, 15, 53 <sup>a</sup>	3, 8	347, 348	53 was originally incorrectly identified as 17 acetate
<i>Solanum balbisii</i>	1	3	229	only 1 reported
<i>S. decasepalum</i>	1	3	349	only 1 reported
<i>S. dulcamara</i>	1, 3, 17, 30, 40, 41, 42, 56, 57, 60	3	227, 235, 242, 350	
<i>S. esculentum</i>	1, 30, 35	3	241	
<i>S. hendersonii</i>	6	4	240	only 6 reported

Source	Pigments	Major pigment patterns (see text)	Ref.	Notes
<i>S. lycopersicum</i>	1, 2, 3	2, 3	351	
<i>Sorbus aucuparia</i>	2, 3, 4, 5, 9, 23, 29	2, 4	225, 333, 352	
<i>Synaspadix petrichiana</i>	1	8	229	only 1 reported
<i>Tabernae montana pentasticta</i>	1	3	229	only 1 reported
<i>Tamus communis</i>	1, 2, 6, 8, 9, 10, 11, 17, 22, 26, 29	3, 4	235, 242, 307	
<i>Taxus baccata</i>	2, 8, 9, 17, 29, 51, 52	2, 4	235, 353, 354	
<i>Trichosanthes cucumeroides</i>	1, 2, 11		355	
<i>Trichosanthes</i> spp.	1	3	356	only 1 reported
<i>Triphasia trifolia</i>	43, 44	4	357	
<i>Vaccinium</i> spp. (blueberry)	complex	2	327	
<i>V. macrocarpon</i>	3, 4, 5, 6, 7, 8, 9, 10, 13, 14, 15, 17, 20, 22, 24, 26, 29, 36, 37	2	327	
<i>V. vitis-idaea</i>	1, 3, 6	4	358	
<i>Viburnum opulus</i>	1, 2, 9, 10, 11, 29	4, 5	235	
<i>Vigna sinensis</i>	2, 5	2	359	
<i>Vitis vinifera</i>	1, 3, 4, 6, 7, 8, 9, 26	2	330	additional epoxides present

## Key

1. Lycopene	(1.11)	17. Lycoxanthin	(5.11)
2. $\beta$ -Carotene	(1.13)	18. $\delta$ -Carotene	(4.4)
3. Lutein	(1.74)	19. Procarotenes	
4. $\alpha$ -Carotene	(1.12)	20. Lutein-5,6-epoxide	
5. $\beta$ -Cryptoxanthin	(1.60)	21. Capsorubin	(1.59)
6. Zeaxanthin	(1.18)	22. $\alpha$ -Cryptoxanthin	(4.1)
7. Violaxanthin	(1.58)	23. $\beta$ -Carotene-5,6-epoxide	
8. Phytoene	(1.36)	24. Neoxanthin	(1.47)
9. Phytofluene	(1.37)	25. Neoxanthin 5,6-epoxide (neochrome)	(5.19)
10. $\zeta$ -Carotene	(1.14)	26. Zeaxanthin 5,6,5',8'-diepoxide [luteoxanthin]	(5.5)
11. $\gamma$ -Carotene	(1.38)	27. Zeaxanthin 5,8,5',8'-diepoxide [auroxanthin]	(1.62)
12. Capsanthin	(1.30)	28. Neurosporene	(2.14)
13. Antheraxanthin	(1.24)	29. $\beta$ -Carotene, 5,8-epoxide (mutatochrome)	(1.44)
14. Mutatoxanthin	(5.4)		
15. Flavoxanthin,	(4.3)		
Chrysanthemaxanthin	(4.2)		
16. Bixin	(5.9)		

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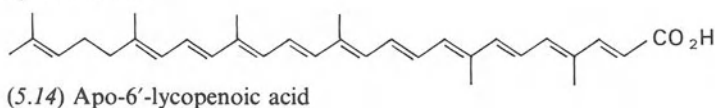
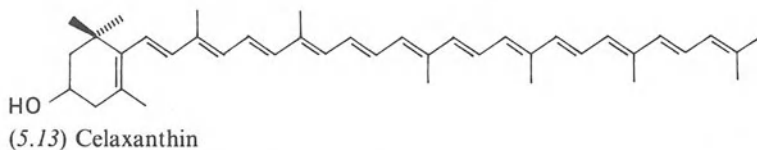
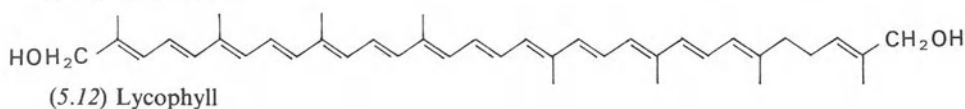
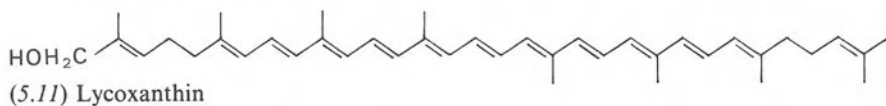
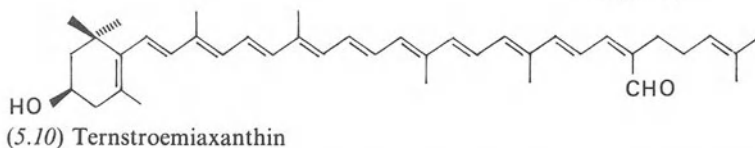
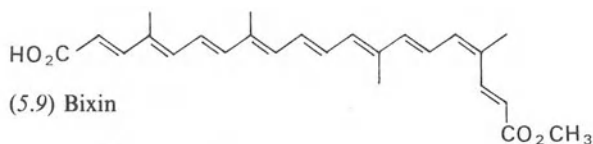
- |   |        |  |        |
|---|--------|--|--------|
| 30. Lycophyll   | (5.12) | 51. Rhodoxanthin   | (4.10) |
| 31. $\beta$ -Carotene,5,8,5',8'-diepoxide (aurochrome)        | (5.7)  | 52. Eschscholtzxanthin   | (1.25) |
| 32. Celaxanthin   | (5.13) | 53. Apo-6'-lycopenoic acid <sup>a</sup>  | (5.14) |
| 33. $\alpha$ -Cryptoxanthin,5,8-epoxide (cryptoflavin)        |        | 54. 5,8-Epoxy-5,8-dihydro-10'-apo- $\beta$ -carotene-3,10'-diol                      |        |
| 34. $\beta$ -Cryptoxanthin,5,8,5',8'-diepoxide (cryptochrome) |        | 55. 3-Hydroxysintaxanthin (3-Hydroxy-7',8',-dihydro-7'-apo- $\beta$ -caroten-8'-one) |        |
| 35. Rubixanthin   | (4.9)  | 56. Lycopene 1,2-epoxide   |        |
| 36. Valenciaxanthin <sup>b</sup>                              |        | 57. $\zeta$ -Carotene 1,2-epoxide  |        |
| 37. Sinensixanthin <sup>b</sup>                               |        | 58. Apo-6'-lycopenal   |        |
| 38. Valenciachrome <sup>b</sup>                               |        | 59. Apo-8'-lycopenal   |        |
| 39. $\beta$ -Citaurin   | (2.23) | 60. 3,4-Dihydrolycopen-16-al   |        |
| 40. Phytoene 1,2-epoxide                                      |        | 61. $\beta$ -Cryptoxanthin-5,6-epoxide   |        |
| 41. Phytofluene 1,2,epoxide (also 1',2'-epoxide)              |        | 62. $\beta$ -Citaurin (8'-apo- $\beta$ -carotene-diol)                               |        |
| 42. Lycopene 5,6-epoxide                                      |        | 63. Loniceraxanthin <sup>b</sup>   |        |
| 43. Semi- $\beta$ -carotenone                                 | (2.23) | 64. Webbiexanthin <sup>b</sup>   |        |
| 44. $\beta$ -Carotenone                                       | (5.18) | 65. 3-Hydroxy-5,8-dihydro-8'-apo- $\beta$ -caroten-8'-al                             |        |
| 45. Mimulaxanthin   | (5.2)  | 66. 5,6-Dihydro- $\beta$ , $\beta$ -caroten-3,3',5,6-tetrol                          |        |
| 46. $\beta$ -Apo-8'-carotenal                                 |        | 67. 5,8-Epoxy-5,8,5',6'-tetrahydro- $\beta$ , $\beta$ -caroten-3,3',5',6'-tetrol     |        |
| 47. $\beta$ -Apo-12'-carotenal                                |        |  |        |
| 48. Persicaxanthin <sup>b</sup>                               |        |  |        |
| 49. $\beta$ -Zeacarotene                                      | (2.12) |  |        |
| 50. $\beta$ -Carotene-5,6,5',6'-diepoxide                     |        |  |        |

### Notes

<sup>a</sup> Originally reported as a methyl ester which is probably an artefact [360].

<sup>b</sup> Structures unknown.

<sup>c</sup> Isocryptoxanthin (4-hydroxy- $\beta$ -carotene) also reported [299].



Early workers reported the presence of unidentified carotenoids in the fruit of numerous species. They are listed in Table 5.9 because a full investigation of these and other species is urgently required.

### (b) *Quantitative distribution*

The quantitative distribution of total carotenoids and  $\beta$ -carotene (which is a measure of vitamin A activity) in a number of fruit is given in Table 5.10. Some of the earlier values of carotene levels in fruit were given in the first edition of this book and are not reproduced here.

### (c) *Localization*

Pigments vary both quantitatively and qualitatively between the mesocarp (pulp) and exocarp (peel) in a number of fruit and this is exemplified in citrus fruit (see Table 5.10). The exocarp is generally the region of highest concentration [252, 263, 393–397] and this is particularly marked in citrus fruit [394, 398] and in apples [361, 399]; 50–75% of the total pigments of an orange

Table 5.9 Some fruit which contain unidentified carotenoids\*

<i>Plant</i>	<i>Ref.</i>	<i>Plant</i>	<i>Ref.</i>
Moraceae		Myrtaceae	
<i>Cannabis sativa</i>	360	<i>Eugenia uniflora</i>	369
Polygonaceae		Solanaceae	
<i>Fagopyrum esculentum</i>	361	<i>Lycium carolinianum</i>	363
Anonaceae		<i>Lycium ovatum</i>	363
<i>Polyalthia</i> spp.	362	<i>Lycopersicon ceraciforme</i>	362
Myristicaceae		<i>Solanum corymbosum</i>	369
<i>Myristica fragrans</i>	363, 364	<i>Solanum pseudocapsicum</i>	363
Rosaceae		Pedaliaceae	
<i>Crataegus crus-galli</i>	365	<i>Sesamum indicum</i>	288
<i>Sorbus aria</i>	366, 367	Caprifoliaceae	
<i>Sorbus suecica</i>	363	<i>Lonicera tatarica</i>	372
Leguminosae		<i>Lonicera xylostium</i>	202, 364,
<i>Azelia cunazensis</i>	368		373, 374
Celastraceae		<i>Viburnum lantana</i>	367, 374,
<i>Euonymus latifolius</i>	367, 369		375
Vitaceae		Cucurbitaceae	
<i>Ampelopsis hederacea</i>	367, 370	<i>Cucurbita pepo</i>	376
Malvaceae		Compositae	
<i>Gossypium hirsutum</i>	369, 371	<i>Helianthus annuus</i>	288

\* Compiled by Karrer & Jucker [84].

is in the peel and the carotenoid levels in apple peel can be five times those in the flesh.

In many fruit, e.g. citrus, peppers and tomatoes, the ripening process involves not only the synthesis of carotenoids but the disappearance of chlorophylls. These changes are accompanied by the change of chloroplasts (carotenoids and chlorophylls) into chromoplasts (carotenoids only) with concomitant profound changes in ultrastructure which can vary in different fruit [400]. For example, grana (thylakoids) disintegrate and are replaced by large numbers of osmiophilic globules (plastoglobuli) and tubular filaments in the case of red peppers (*Capsicum annuum*) [401–407], *Solanum capsicum* [401], *Physalis alkekengi* [401], *Solanum capsicastrum* [140, 141, 401], *Cucurbita pepo* [410] and *Rosa rugosa* [411]. In mutants of *Capsicum annuum* which synthesize little or no carotenoids fibrils are also absent or much reduced in size. Inhibition of formation of cyclic carotenoids by CPTA (see Chapter 2) results in the inhibition of fibril formation and the deposition of lycopene crystalloids within the plastid [407A].

In Valencia oranges osmiophilic globules are also formed from the internal generating system together with membranes which develop from invagination of the inner part of the limiting membrane of the chloroplast [408]. One

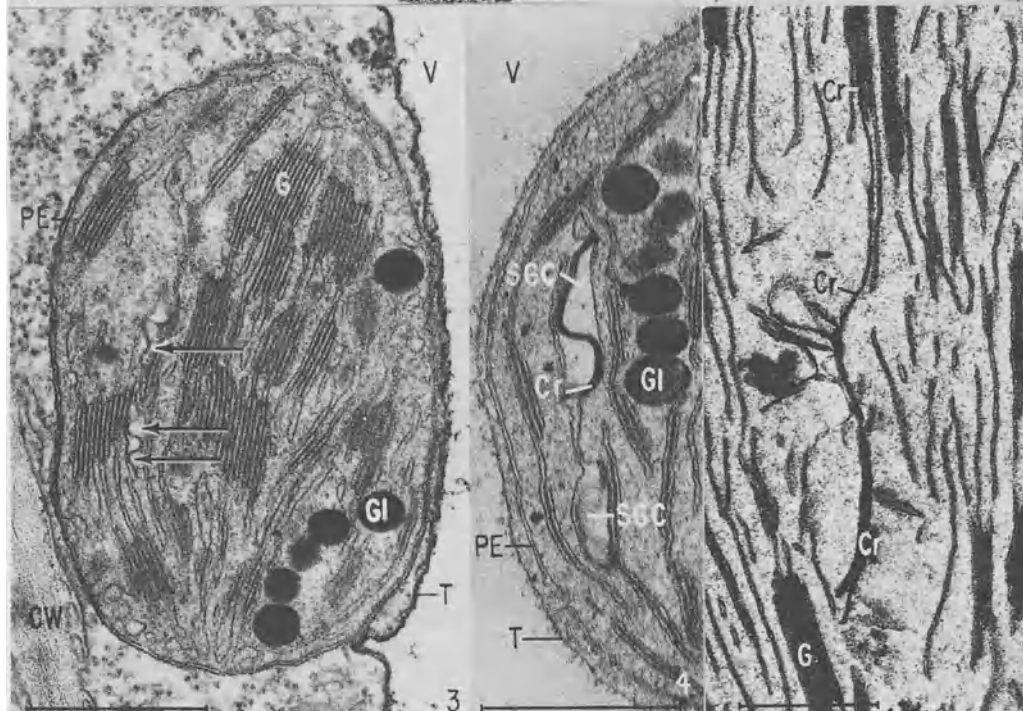
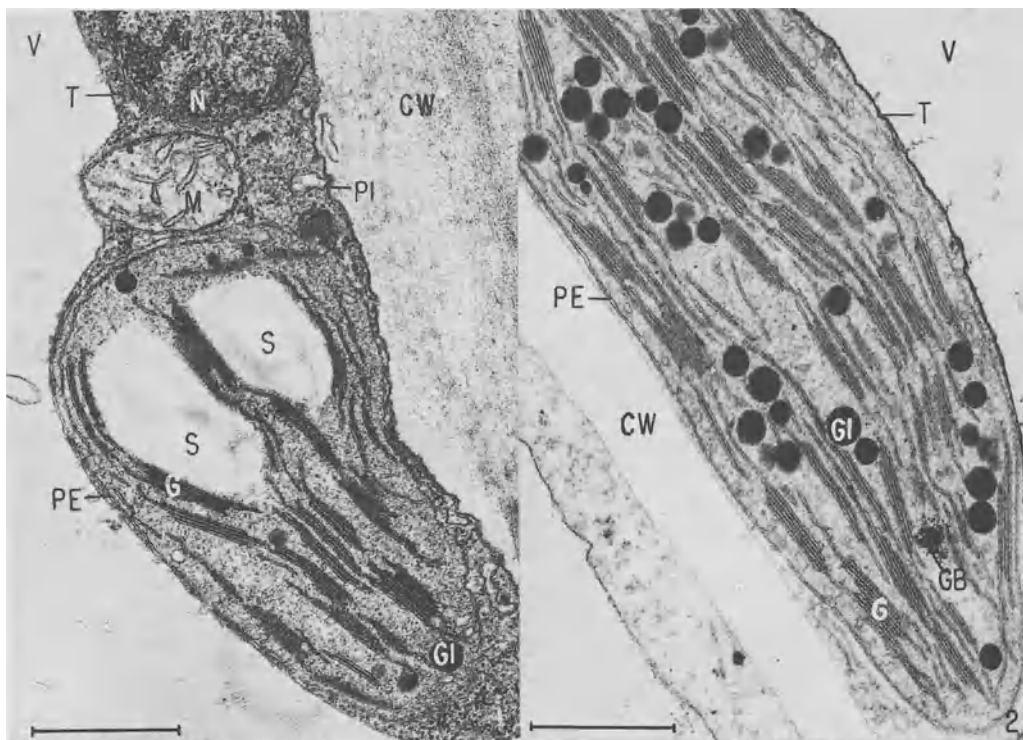


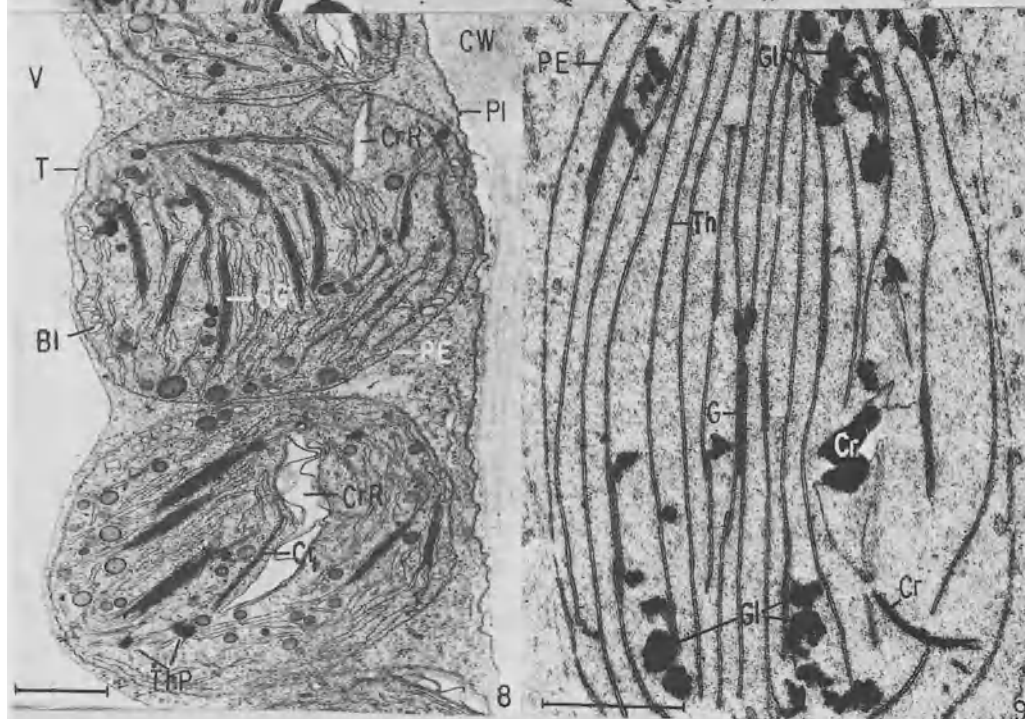
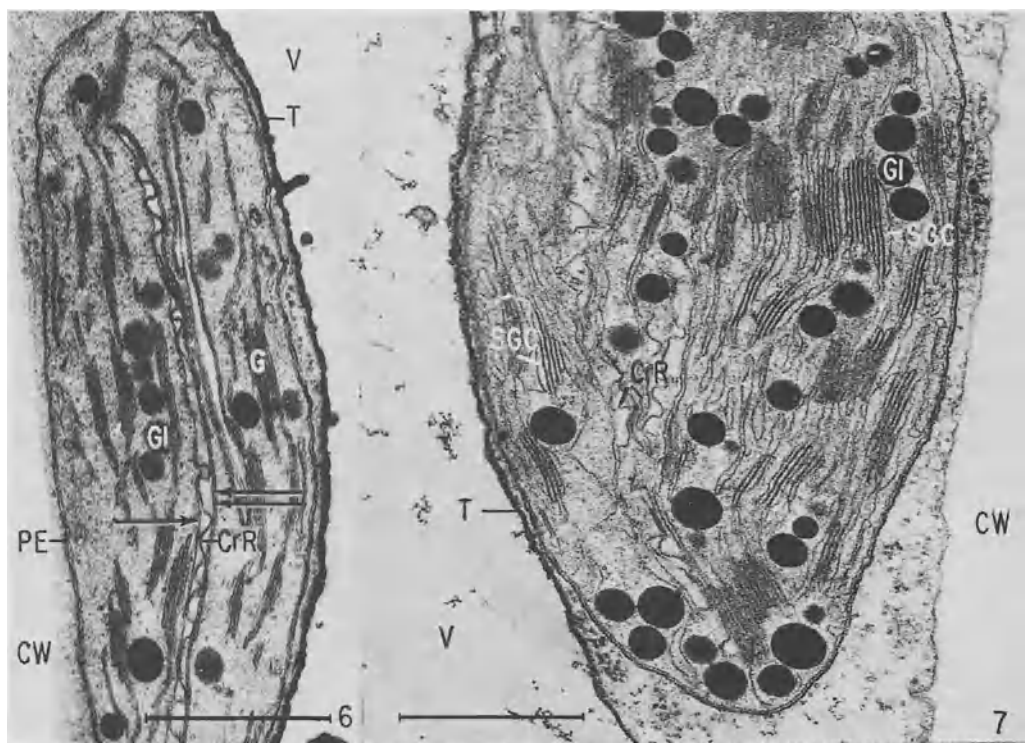
*Table 5.10* Total carotenoid and  $\beta$ -carotene level of some fruit

Species	Carotenoid mg/kg fresh weight		Ref.
	Total pigments	$\beta$ -Carotene	
<i>Aglaonema commutatum</i>	120	18.3	377
<i>Ananas comosa</i> (pineapple)	—	2.6–7.4	378
<i>Arum maculatum</i>	200	22.7	235
<i>Aronia melanocarpa</i>	7.5–12.2		
<i>Atropa belladonna</i>	18	4.1	235
<i>Berberis</i> spp.	—	0.17–5.9	238
<i>Capsicum annuum</i> (green)	9.0–11.2	1.2–1.5	248
(red)	127–248, 855	11.6–33, 105	235, 244, 378A
(yellow)	22.4	0.224	378A
(orange)	24.9	0	378A
(white)	0.69	—	378A
<i>Carica papaya</i>	13.8	4.1	252
(red)	4.2	0.2	222
(yellow)	3.7	0.15	222
<i>Citrullus vulgaris</i> (red)	20.9–61.7	0.41–5.96	256
(orange)	33.7	1.4	257
<i>Citrus limonium</i> (peel)	10	—	379
<i>Citrus nobilis</i> (mandarin)	—	2.5	381
(pulp)	27	1.11	249
(peel)	186	0.74	380
<i>Citrus paradisi</i> (pulp)	8.2	2.2	264, 265
(ruby red: peel)	10.4	0.75	265
<i>Citrus sinensis</i> Navel–peel	23	0.115	269
pulp	67	0.1	269
juice	4–7 <sup>a</sup>	0.032	381
Nagpur–pulp	14.4	0.01	252
peel	38.5	0.45	250
Shamouti–juice	6–10	0.20 <sup>a</sup>	382
Valencia–pulp	24	0.26	269
peel	98	0.29	269
juice	10–15	0.30 <sup>a</sup>	382
<i>Cotoneaster</i> spp.	1.5–16	1.12	235
<i>Crataegus azarolus</i>	—	5 <sup>b</sup>	274
<i>Crataegus</i> spp.	4–25	2.8–5.8	235
<i>Cucumis melo</i> (cantaloup)	20.9–61.7	0.41–5.96	256
<i>Dioscorea</i> spp. (yam)	—	4.34	382A
<i>Diospyros kaki</i> (persimmon)	21.6–97.9	1.2–7.5	284
	54	3.9	248, 283
	29–78	1.2–7.5	383
<i>Ficus carica</i> (Fig)	8.5	0.49	248
<i>Fragaria chiloensis</i> (strawberry)		1.5	384
<i>Hippophae rhamnoides</i>	52	1.6	253
		4.8–9.1 <sup>b</sup>	385
<i>Lonicera</i> spp.	18	1.3	253
<i>Malpighia punicifolia</i> (pulp)	57.9	26.2	307

<i>Species</i>	<i>Carotenoid mg/kg fresh weight</i>		<i>Ref.</i>
	<i>Total pigments</i>	<i>β-Carotene</i>	
<i>Mangifera</i> spp.–Badami	108–154	12–140	380
Baneshan	23.7	—	386
Dashehan	90.6	—	386
Malgoa	21.8–25.7	12	387
<i>Musa cavendishi</i>	0.24	0.8	313
<i>Passiflora edulis</i> (juice)	6.6	2.7	316
<i>Persea americana</i> (avocado)	10–14	0.4–0.6	317, 388
<i>Physalis alkekengi</i> (sepals)	8.683	86.5	307
(berries)	573	3.85	307
<i>Prunus armenica</i> (apricot)	35	21	248, 389
<i>P. domestica</i> (prune)	—	3.9	244
<i>P. persica</i> (peach)	27	2.7	248, 389
<i>Pyracantha</i> spp.	1–15	trace–4.95	235
<i>Pyrus</i> spp. (cherry)		5–11	384
<i>Pyrus baccata</i> (crab apple)	19.8	4.2	361
<i>Pyrus communis</i> (pear), peel	0.3–1.28		384
mesocarp	5.6		384
<i>Pyrus malus</i> (apple), whole fruit	54.9–126.1	1.98–76.3	361
peel	5.6		384
mesocarp	0.9–5.4		384
<i>Pyrus prunifolia</i> (plum)	2.3	0.12	361
<i>Punica granatum</i> (pomegranate)	0.16–0.47	1.431	248, 390
<i>Ribes nigrum</i> (blackcurrant)	0.8 <sup>b</sup>		235
<i>Rosa acicularis</i>		5	391
<i>R. canina</i>	25	16.5	232
	11.92	3.0	365
	—	3.2 <sup>b</sup>	392
<i>R. moyesii</i>	224	53.7	235
<i>R. rubrifolia</i>	88	50.1	235
<i>Rubus procerus</i> (blackberry)	5.9	0.56	248
<i>Sambucus nigra</i>	16	3.0	235
<i>Solanum dulcamara</i>	90	55	235
<i>Sorbus aucuparia</i>	120	46.8	235
<i>Taxus baccata</i> (whole berry)	10	0.4	235
<i>Viburnum opulus</i>	607	8.1	235
<i>Vaccinium macrocarpon</i> (cranberries)	5.8	0.3	248
<i>Vaccinium</i> spp. (blueberry)	2.7	—	248
<i>Vitis vinifera</i>	1.8	0.58	248
<i>Tamus communis</i> (berries)	529	23	235
<i>Ziziphus jujuba</i>	—	28	393

*Notes*<sup>a</sup> mg/l juice.<sup>b</sup> Based on an assumed 90% water content.





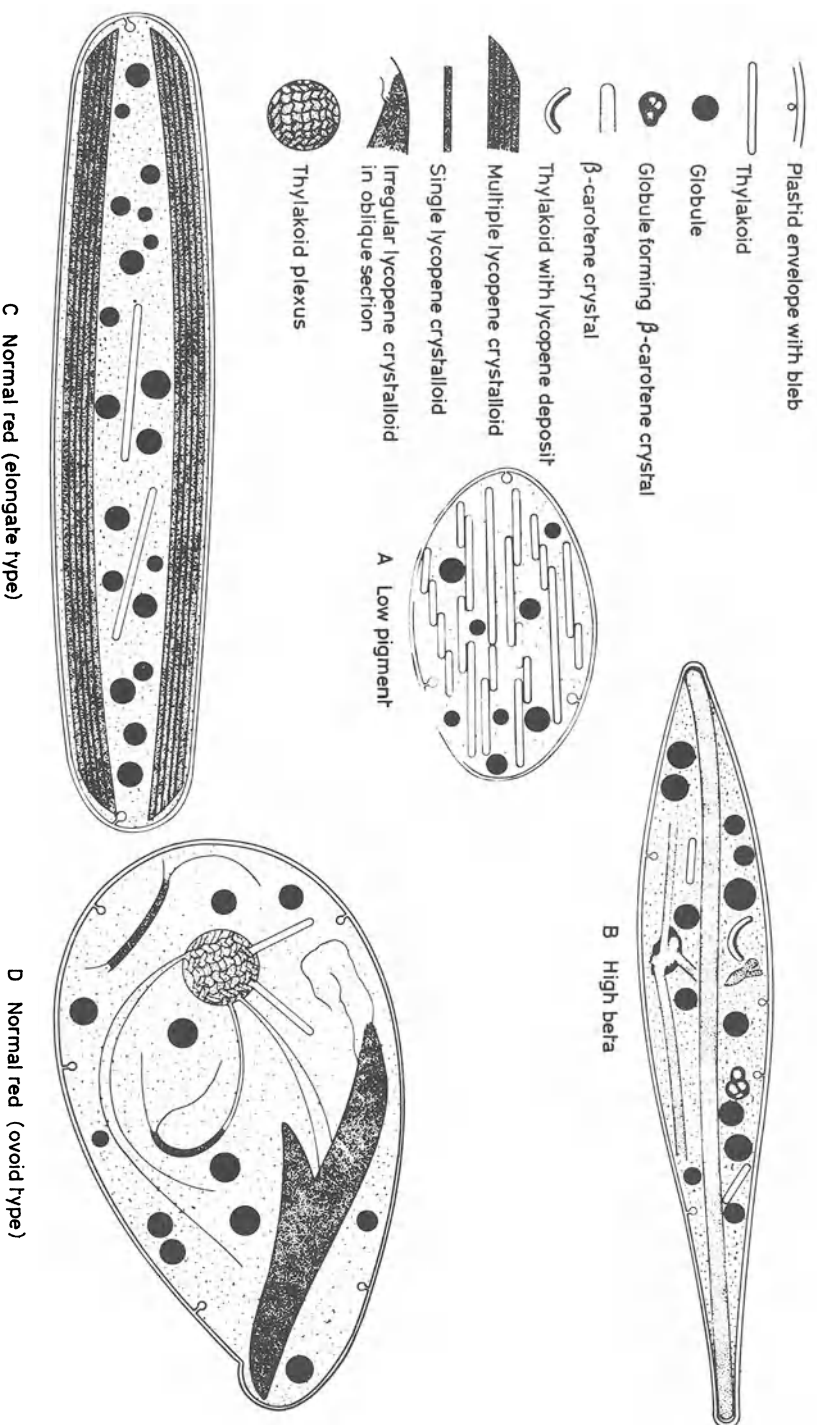
assumes that the lipophilic carotenoids accumulate in the osmiophilic globules, although it has been suggested that carotenoids are complexed with protein in the fibrils [401]. Other chromoplasts contain essentially only osmiophilic globules, as are seen frequently in flower petals (5.1.2(c)). Examples include orange, yellow and white varieties of *Capsicum annuum* [402] and navel oranges [408]. Red tomato fruit produce two types of chromoplast [406, 402–416]; one type is localized in the jelly part of the pericarp and contains osmiophilic globules rich in  $\beta$ -carotene; the other is found mainly in the outer part of the fruit and contains as well as osmiophilic globules numerous sheets (crystalloids) of lycopene (Fig. 5.2); these differences have been emphasized by pigment analysis [417]. The chromoplasts of low pigment and high  $\beta$ -tomato strains have also been examined and are also indicated

Fig. 5.2. Electron micrographs of developing chromoplasts in normal red tomato (kindly provided by Dr W. M. Harris) [415]

Key: B1, bleb; Cr, pigment crystalloid; CrR, pigment crystalloid remnant; CW, cell wall; ER, endoplasmic reticulum; G, granum; GB, granular body; GG, giant granum; Gl, globule; M, mitochondrion; N, nucleus, PE, plastid envelope; Pl, plasmalemma; R, ribosome; S, starch grain; SGC, swollen granum compartment; Str, stroma; T, tonoplast; Th, thylakoid, ThP, thylakoid plexus; V, vacuole. The scale line represents 0.5  $\mu$ m.

(a–e) Early stages of chromoplast development. (a) Chloroplast from mature green fruit. Starch grains and a few globules are present along with grana and other internal membranes. Glutaraldehyde-osmium fixation. X44 000. (b) Plastid from green area of turning fruit just beginning to change. Note increase in number of globules and the granular body. Glutaraldehyde-osmium fixation. X42 500. (c) Transition plastid from turning fruit showing beginnings of lycopene crystalloid formation (arrows). Glutaraldehyde-osmium fixation X54 400. (d) Transition plastid from turning fruit showing pigment crystalloid formation within the granal compartment. Note the swollen granal compartment and undulating crystalloid. Glutaraldehyde-osmium fixation. X60 400. (e) Transition plastid from turning fruit at a developmental stage comparable to (d). Note that the developing pigment crystalloid is relatively straight and the thylakoid is not swollen (the thylakoid membranes are obscured by the pigment crystalloid). Glutaraldehyde-KMnO<sub>4</sub> fixation. X41 900.

(f–i) Transition stages between chloroplasts and chromoplasts from fruits at the turning stage of ripening. (f) Plastid showing development of pigment crystalloids in elongated granal compartment; a single arrow indicates outer compartment membrane (end granal membrane) of adjacent compartment which contains lycopene crystalloid. Glutaraldehyde-osmium fixation. X55 200. (g) Plastid showing breakdown of grana structure. Note swollen grana compartments and pigment crystalloids. Glutaraldehyde-osmium fixation. X54 800. (h) Two plastids showing 'giant grana' and other elongated membranes. The darkly staining partition (Cr<sub>1</sub>) may represent early stages of lycopene deposition. Note blebs from inner element of the plastid envelope (PE) and the thylakoid plexus (ThP). Glutaraldehyde-osmium fixation. X27 800. (i) Plastid showing elongated thylakoids and reduction in granal organization. The form of the globules is distorted with this fixation. Glutaraldehyde-KMnO<sub>4</sub> fixation. X41 900.



*Fig. 5.3.* Diagrammatic representation of mature chromoplasts in three pigment lines of tomato: normal (red), high  $\beta$  and low pigment [redrawn from 414].

diagrammatically in Fig. 5.3. In the delta strain of tomatoes (large amounts of  $\delta$ -carotene, see next section) a rather similar breakdown of chloroplasts and formation of chromoplasts has been observed [405]. Carotenoid-rich chromoplasts can also be formed from colourless vesicles or proplastids rather than from chloroplasts, as in *Asparagus officinalis* [418] and *Convallaria majalis* [419].

#### (d) *Mutants*

*Tomatoes.* Probably tomatoes and citrus fruit are the most important carotenogenic fruit and it is on the former that the most far-reaching mutant studies on carotenoids have been carried out. The major observations are summarized in Table 5.11. Normal red tomatoes possess the dominant allele  $r^+$  whilst yellow tomatoes are homozygous for the recessive allele  $r$ . The  $r^+r$  gene also controls the amount of pigment formed, the  $rr$  genotype synthesizing only about 5% (4  $\mu\text{g/g}$  fresh weight) of the pigment formed by the  $r^+r^+$  genotype [420]. *Apricot* tomatoes, which are homozygous for the recessive allele  $at$ , have reduced synthesis of the lycopene (acyclic) series of pigments whilst the synthesis of  $\beta$ -carotene is unaffected [421]. A third gene  $hp^+hp$  also controls quantitatively pigment synthesis; the recessive allele  $hp$  increases total synthesis by 100% [422]. Tangerine tomatoes are homozygous for the recessive allele  $t$ , whilst red tomatoes carry the dominant allele  $t^+$  [430]. The presence of  $t$  causes the accumulation of the poly-*cis*-isomers, prolycopene (1.55) and pro- $\gamma$ -carotene [431, 432] at the expense of the all *trans*-isomer although traces of pro- $\gamma$ -carotene are present in  $t^+$  fruit [432A]. The effects of  $r$  and  $t$  are both reinforced by  $at$ , for in the yellow apricot phenotype, lycopene (1.11) synthesis is completely repressed and  $\beta$ -carotene (1.13) synthesis reduced whilst in the tangerine-apricot phenotype synthesis of procarotenenes is stimulated [419]. If *L. esculentum* is back-crossed with a *L. hirsutum* hybrid an orange phenotype is obtained in which  $\beta$ -carotene replaces lycopene. These fruit (high  $\beta$ ) are either homozygous or heterozygous for the dominant allele  $b^+$  whilst normal fruit are homozygous for the recessive  $b$  [425]. A number of other crosses have produced tomatoes with high  $\beta$ -carotene levels [433–438]. Expression of the  $b^+b$  gene is partly regulated by an independently inherited modifier  $mo_B^+mo_B$  [439]. In the presence of the allele  $mo_B^+$  in either the homozygous or heterozygous form, the level of  $\beta$ -carotene is reduced by about 50% whilst that of lycopene is increased to about the same amount. A similar gene, which may indeed be identical to the  $b^+b$  gene, is present in an orange-fruited *L. pimpinellifolium* from the Galapagos Islands [440] and in *L. hirsutum*  $\times$  *L. chilense* [437], *L. esculentum*  $\times$  *L. hirsutum* and *L. esculentum*  $\times$  *L. chilense* [441]. The delta strain of tomatoes, which accumulates large amounts of  $\delta$ -carotene (4.4) at the expense of lycopene, carries the delta allele  $del$  [442] which is either incompletely dominant or dominant but affected by a modifier gene. The

Table 5.11 Carotenoid distribution in various tomato mutants

Phenotype	Genotype	Main pigments and colourless polyenes present	Concentration (mg/g fresh wt.)		Ref.
			Total polyenes	Pigments only	
Red <sup>a</sup>	<i>r<sup>+</sup>r<sup>+</sup></i>	1, 2, 3, 4, 5	87	50	423
High pigment	<i>hp hp</i>	1, 2, 3, 4, 5	88	69	423
Tangerine	<i>r<sup>+</sup>r<sup>+</sup>tt</i>	2, 3, 4 <sup>d</sup> , 5 <sup>d</sup> , 6, 7 <sup>d</sup> , 11	158	90	423, 424
Yellow	<i>rr t<sup>+</sup>t<sup>+</sup></i>	1, 2, 4, 5	4	2	424
Yellow-Tangerine	<i>rr tt</i>	1, 2, 4, 5, 7	34	14	421
Apricot	<i>at at</i>	1, 2, 4, 5	13	11	424
Yellow-Apricot	<i>rrt<sup>+</sup>t<sup>+</sup>atat</i>	2, 4, 5 (trace)	2	1	421
Tangerine-Apricot	<i>r<sup>+</sup>r<sup>+</sup>t<sup>+</sup>t<sup>+</sup>atat</i>	1, 2, 4, 5, 6, 7	29	16	421
Yellow-Tangerine-Apricot	<i>rrttatat</i>	4, 5	10	0	421
Ghost	<i>ghgh</i>	1, 4, 5	295	1	425
Intermediate $\beta$	<i>b<sup>+</sup>b<sup>+</sup></i>	1, 2, 3, 4, 5	50	46	423
High $\beta$ (orange)	<i>b<sup>+</sup>b<sup>+</sup>mo<sub>B</sub>mo<sub>B</sub></i>	1, 2, 3, 4, 5	80	70	423, 426
Delta <sup>b</sup>	<i>del<sup>+</sup>del<sup>+</sup></i>	1, 2, 3, 4, 5, 7, 8, 9, 10	84	68	423
High-Delta	<i>del<sup>+</sup>del<sup>+</sup> hphp</i>	1, 2, 3, 4, 5, 7, 8, 9, 10	55	46	423
Rin <sup>c</sup>	<i>nn</i>	1, (traces) 4, 5			427, 428

## Key

1. Lycopene	(1.11)	7. $\zeta$ -Carotene	(1.14)
2. $\beta$ -Carotene	(1.13)	8. $\alpha$ -Carotene	(1.12)
3. $\gamma$ -Carotene	(1.38)	9. $\delta$ -Carotene	(4.4)
4. Phytoene	(1.36)	10. Neurosporene	(2.14)
5. Phytofluene	(1.37)	11. Pro- $\gamma$ -carotene	
6. Prolycopene (poly <i>cis</i> )	(1.55)		

## Notes

<sup>a</sup> Also 1,2-epoxides of phytoene, phytofluene,  $\zeta$ -carotene and lycopene 5,6-epoxide [224, 225].

<sup>b</sup> Also epoxides of lycopene, and 1',2'-epoxides of  $\gamma$ - and  $\delta$ -carotenes [429].

<sup>c</sup> Lycopene synthesis is almost completely inhibited.

<sup>d</sup> Present as the 9-*cis* isomers [429A].

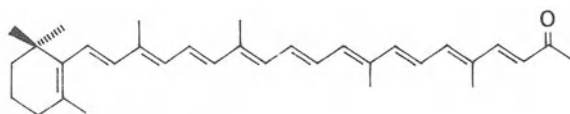
ghost phenotype appears spontaneously in lines carrying red fruit. Seedlings homozygous for the recessive allele *gh* germinate with green cotyledons which rapidly lose chlorophyll as they grow. When normal scions are grafted on to ghost stock fruit are produced which are milky white when unripe and yellowish when ripe. This yellow colour is not due to carotenoids for the fruit contain only the colourless phytoene (1.36) in large amounts. The *gh<sup>+</sup>gh* gene also controls carotenoid synthesis in leaves; plants carrying the recessive *gh* also accumulate only phytoene [424]. The reduced chlorophyll content of such leaves is almost certainly a secondary effect caused by the



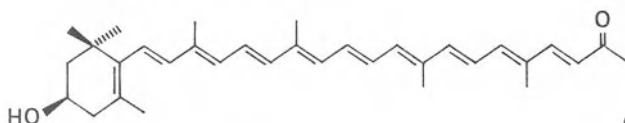
photodestruction of chlorophyll in the absence of coloured carotenoids (see Chapter 3).

A lutescent gene delays the appearance of lycopene in ripening fruit for up to two weeks. During this time the fruit behave as *r* segregants but quite suddenly, within 24–30 hours, they rapidly redden as lycopene synthesis is switched on [442]. The mode of action of this 'timing gene' is unknown. Both chlorophyll and lycopene are present in the ripe fruit of a dirty-red mutant. Carotenoid levels are normal whilst chlorophyll levels instead of being zero are about 30% of that in unripe green fruit; this effect is controlled by a single recessive gene allelic to the green flash (*gf*) mutant [443]. A recessive mutation sherry (*sh*) produces fruit resembling yellow (*rr*, *t<sup>+</sup>t<sup>+</sup>*) fruit but containing rather more carotenoid. However, it is non-allelic to the yellow apricot and tangerine genes [444]. A variety ES<sub>24</sub> carrying the recessive allele *u* (uniform ripening) produces less lycopene than the strain Tecumseh carrying the dominant *u<sup>+</sup>*; however F<sub>2</sub> studies indicated that lycopene loss could not be assigned to the *u<sup>+</sup>u* gene [437]. Carotenoid distribution in a number of tomato crosses from the USSR has been reported [445, 446]. It is claimed from studies on crosses between *L. esculentum* and *L. chilense* that pigment stability was not found before F<sub>7</sub> [447]. Attempts to produce tomatoes acceptable to the public but with high  $\beta$ -carotene content have only been partly successful (see e.g. [448]).

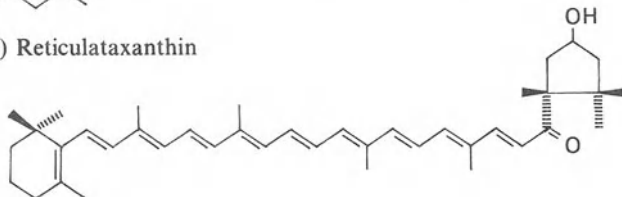
*Citrus*. A number of citrus hybrids with deep red flavedos contain large amounts of the apocarotenoids such as  $\beta$ -citraurin (1.26) and  $\beta$ -apo-8'-carotenal [449]. One such is the Sinton citrangequat, a trigeneric hybrid of the oval Kumquat (*Fortunella margarita*) with the Rusk citrange (*Poncirus trifoliata* x *Citrus sinensis*). The large amounts of citranaxanthin (5.17) and



(5.15) Citranaxanthin



(5.16) Reticulataxanthin



(5.17) Cryptocapsin

reticulataxanthin (5.16) previously reported [450, 451] are apparently artefacts produced from the naturally occurring apocarotenoids in the presence of acetone and alkali [449]. Another strain is the cross between Orlando tangelo and clementine (Robinson) which produces mainly  $\beta$ -citraurine (8'-apo- $\beta$ -caroten-3-ol) [273]. Pink and red grapefruit synthesize lycopene [452, 453] as does Sarah, a pink bud sport of Shamouti (Jaffa) orange [454].

*Peppers.* A mutant of *Capsicum annuum* which produces yellow fruit has been known for some time [455]; it produces essentially the carotenoids characteristic of chloroplasts [243, 402, 407]. The mutation has clearly blocked the additional synthesis of  $\beta$ -carotene and its further metabolism into derivatives which include capsorubin (1.59) and capsanthin (1.30) (Table 5.12).

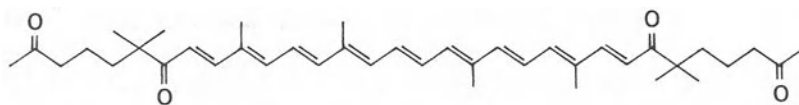
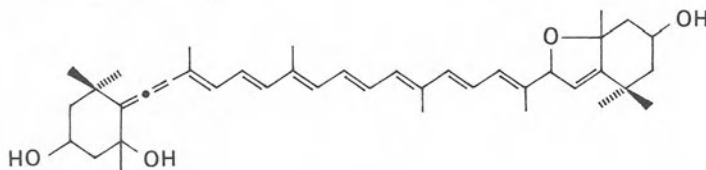
Table 5.12 Carotenoid distribution in ripe fruit of colour variants of *Capsicum annuum* (Adapted from [378]) (Expressed as percentage of total pigment recovered)

Pigment	Red	Orange	Yellow
$\beta$ -Carotene (1.13)	12.3	—	1.0
$\beta$ -Carotene 5,6-epoxide	3.1	—	—
Hydroxy- $\alpha$ -carotene (?)	—	—	5.7
$\beta$ -Cryptoxanthin (1.60)	7.8	—	—
Cryptocapsin (5.15)	5.0	8.0	—
Lutein (1.74)	—	—	28.1
Zeaxanthin (1.18)	6.5	7.3	15.9
Antheraxanthin (1.24)	9.2	10.6	31.3
Violaxanthin (1.58)	9.8	14.9	—
Capsanthin (1.30)	31.7	35.4	—
Capsanthin-5,6-epoxide	4.2	6.1	—
Capsorubin (1.59)	7.5	14.3	—
Neoxanthin (1.47)	2.0	2.7	20.0

An orange variant appears to have the  $\beta$ -carotene/capsanthin pathway drastically reduced quantitatively [378]. Mutants are also known with no carotenoids present in their fruit although the leaf carotenoids are normal [402]. It has been suggested that in this case mutation has resulted in the formation of a faulty regulator gene which cannot combine with its inducer, thus some or all of the structural genes concerned with carotenogenesis remain suppressed [456]. Treatment of pepper seeds with nitrosomethylurea is said to produce variants with raised carotenoids in the fruit [457].

*Papaya.* The red fleshed variety differs from the usual yellow fleshed varieties by a single gene and by synthesizing large amounts of lycopene (251). It would appear that the gene is similar to the  $r^+r$  gene in tomatoes (see Table 5.11).

*Watermelon.* Orange fleshed watermelons differ from the normal red type

(5.18)  $\beta$ -Carotenone

(5.19) Neochrome

in synthesizing  $\beta$ -carotene rather than lycopene [257]. A more recently isolated *crimson* type contains more lycopene, phytoene (1.36) and phytofluene (1.37) than the normal strains whilst the  $\beta$ -carotene levels are lower [458].

### 5.1.5 RIPENING OF FRUIT

#### (a) *Patterns of ripening*

In fruit such as the tomato in which ripening is accompanied by the conversion of chloroplasts into chromoplasts there is a rapid synthesis of characteristic fruit carotenoids associated with a simultaneous fall in chlorophyll and plastid carotenoids [459]. The change begins about 47 days after anthesis and is complete within 55 days. In mutants *nin* (yellow) and *nor* (pink) the change is much slower and less complete; for example, even 95 days after anthesis some grana, characteristic structures of chloroplasts, are still present in the chromoplasts [460].

Apart from the well-documented case of tomatoes [459–465], similar changes have been demonstrated in orange flavedo [466–468], juice [469, 470] and pulp [468, 471, 472], as well as in pumpkins [473], rose hips of various species [378, 474], mangoes [475–479], grapes [479], musk melons [480] and various citrus fruit other than those previously mentioned [395]. It has been reported that such increases are not observed in Golden Delicious and Grimes Golden apples [481] but they have been observed in other studies [482] and in particular in Cox's Orange pippin where the increase is marked at the climacteric [483]. In Golden Delicious grown in the US there is a minimum in September but the levels then increase [484]. It is interesting that carotenoid levels in apples depend mainly on the properties of the root stock [485]. No changes have been reported in the carotenoids of banana skins during ripening [486]. In grapes carotenoids disappear during ripening presumably as the chloroplasts disintegrate rather than transform into chromoplasts [487]. There are obviously quantitative changes during ripening and this is

well illustrated by comparing the carotenoids of unripe and ripe peppers (Table 5.13) [243]. The distribution in unripe fruit is very similar to that in the leaves but the situation in ripe fruit shows the massive synthesis

Table 5.13 The major carotenoids of leaves, unripe and ripe fruit of *Capsicum annum* var. *lycopersiciforme rubrum* [243]

Pigment	Amount (mg/100 g fresh wt.)		
	Leaves	Unripe fruit	Ripe fruit
$\beta$ -Carotene (1.13)	7.92	0.095	2.35
$\beta$ -Cryptoxanthin (1.60)	0.45	0.027	1.10
Lutein (1.74)	13.99	0.276	—
Zeaxanthin (1.18)	—	—	1.75
Antheraxanthin (1.24)	1.14	0.031	0.99
Violaxanthin (1.58)	8.27	0.042	0.70
Neoxanthin (1.47)	5.66	0.058	—
Capsanthin (1.30)	—	—	9.60
Capsorubin (1.59)	—	—	1.46

of capsanthin and related pigments (see also Table 5.12). In the case of oranges there is a greater synthesis of xanthophylls relative to carotenes during ripening [273, 467, 488–490]; these observations contradict the much earlier reports that during ripening carotenes increase more than xanthophylls [324, 394]. The carotene:xanthophyll ratio in pears in early summer is 0.41; it rises to 0.61 in high summer and falls to 0.41 in the autumn. The same situation exists in frost-resistant and low frost-resistant strains [491]. Ripening of apricots is accompanied by a very substantial synthesis of  $\beta$ -carotene (1.13) whilst the other components increase to a much smaller degree; in peaches three pigments,  $\beta$ -carotene, lutein (1.74) and violaxanthin (1.58), increase markedly during ripening of the fruit, whilst  $\beta$ -cryptoxanthin (1.60) and neoxanthin (1.47) increase only very slightly [322]. Carotenoids increase very rapidly in the peel and flesh of pineapple just at the ripe stage [492].

Although in most carotenogenic fruit chlorophyll disappears rapidly as carotenogenesis takes over, its disappearance is not essential; for example there are tomato and pepper mutants (Section 5.1.3.(d)) in which considerable amounts of chlorophyll are present along with carotenoids in the mature fruit. Furthermore, in grapefruit active carotenogenesis occurs before chlorophyll begins to disappear [266, 453]; indeed in the flavedo of marsh seedless grapefruit there is no further synthesis of coloured carotenoids during ripening after all the chlorophyll has disappeared, although there is a marked synthesis of phytoene [266].

Many fruit are autonomous from the point of view of carotenogenesis; this is well known through the common habit of ripening tomatoes after harvesting. A stored tomato can synthesize up to 1.2 mg lycopene per day

[493], but vine-ripened tomatoes generally contain more pigment than those ripened in storage [494–498]. Other fruit which synthesize carotenoids on storage include mangoes [499, 500], peppers [501, 502] and mandarin oranges [503] whilst apples generally do not [484]. There is one report that isolated discs of the immature pericarp of *Capsicum annuum* when cultured aseptically will ripen and synthesize the characteristic pepper carotenoids [504].

The reason for the marked quantitative changes in carotenogenesis during the ripening of some fruit is not yet fully explained. However, in peppers the permeability of the developing chromoplast to acetate and mevalonate is greatly increased compared to that of the parent chloroplast which is almost zero [504A].

### (b) *Oxygen*

Ripening in the absence of oxygen, i.e. in an atmosphere of N<sub>2</sub> or CO<sub>2</sub>, inhibits carotenoid synthesis in tomatoes, *Physalis alkekengi* [505] and in various citrus fruit [506].

### (c) *Light*

The well known domestic habit of ripening commercial tomatoes in cupboards makes it clear that with this fruit, at least, light is not essential for carotenogenesis. Indeed if fruit on the vine are allowed to mature in complete darkness they become red although they are white when immature [507], but they contain less carotenoid than those ripened in light [506, 508]. However, illumination of dark-grown, dark-ripened tomatoes of red, high- $\beta$  or apricot genotypes has no effect on carotenoid type or synthesis. It is interesting that in tangerine tomatoes matured in the dark there are only poly-*cis* pigments and no all-*trans* neurosporene or lycopene, which exist to some extent in the light-ripened fruit, probably owing to photoisomerization of the naturally formed poly-*cis* pigments [507]. Presumably immature chloroplasts with no chlorophyll can develop into fully biosynthetically competent chromoplasts either in light or darkness. Furthermore, the switch which triggers off carotenogenesis is not light as it is in some micro-organisms (see Chapter 8). In several cases, e.g. golden tomatoes, Elberta peaches, Humbolt nectarines and Royal apricots, synthesis would appear to be greater in the dark [509]. Much of the older literature is contradictory because of the problem of adequate control of light and temperature; as indicated in the next section temperature can have a profound effect on carotenogenesis.

As tomatoes grown in greenhouses often contain less carotenoid than those grown outside [494, 506, 510–513] it has been suggested that this is due to a stimulatory effect of ultra-violet light. However, ultra-violet light inhibits carotenogenesis in golden varieties of tomatoes [508] and ultra-violet irradiation of green (unripe) tomatoes inhibits loss of chlorophyll and

appearance of carotenoids; the situation seems to be reversed with mangoes [500].

The intervention of phytochrome in carotenoid synthesis in tomatoes may also explain some earlier contradictory results; red light stimulates and far-red light inhibits synthesis compared with dark controls, and far-red can eliminate the stimulatory activity of red light [514].

#### (d) *Temperature*

It is well known that a temperature above 30°C inhibits ripening in tomatoes [494, 515, 516] but only relatively recently was it shown that the effect was only on lycopene and its congeners and not on  $\beta$ -carotene synthesis [517, 518, 518A]. This is interesting in relation to a report of two types of chromoplasts in tomatoes which contain mainly lycopene and  $\beta$ -carotene respectively (see Section 5.1.4.(d)). It is also clear that the enhanced  $\beta$ -carotene synthesis which occurs in tomatoes in which the *hp* (high pigment) factor is introduced is not temperature-sensitive and thus the *hp* effect is a quantitative one, on 'normal'  $\beta$ -carotene and lycopene synthesis [423]. On the other hand, in the presence of gene *b* the greatly enhanced synthesis of  $\beta$ -carotene, which is at the expense of lycopene, is temperature-sensitive [423]; in this mutant the lycopene pathway is taken through to  $\beta$ -carotene. The optimal temperature for lycopene synthesis is said to be 16–21°C. Exposure of fruit to temperatures higher than 32°C has no permanent deleterious effect on lycopene synthesis because fruit held at high temperatures and subsequently transferred to a temperature below 32°C immediately begin to synthesize the pigment [519]. This explains why tomatoes exposed to wide temperature fluctuation on the vine will ripen normally if the night temperature drops below 30°C [520]. Temperature also has an effect on speed of ripening, a diurnal regime of 17.8°/25.6°C allows full coloration to develop within 7 days of onset of ripening whilst 7.2°/18.3°C requires 14 days; a low-temperature environment e.g. 2.8°/13.9°C adversely affects the amount of lycopene synthesized [521]. Lycopene synthesis in red blush grapefruit and red blood oranges is also temperature-sensitive [522, 523] but in the case of the grapefruit higher temperatures stimulate lycopene synthesis [523]. The optimum temperature for carotenogenesis in oranges is 15–25°C [524, 525]. Temperature has no qualitative effect on carotenoid synthesis in mangoes [476].

#### (e) *Nitrogen metabolism*

In ripening apples there is a positive correlation between the carotenoid levels and total nitrogen in the peel, and the carotene levels appear to be highest in apples from trees with the highest levels of leaf nitrogen [482].

(f) *Soil nutrients*

Recent reports indicate that potassium deficiency lowers lycopene levels in tomatoes [526, 527] whilst cobalt increases carotenoid levels in the same fruit [528]. An early investigation suggested that variation in mineral nutrients had no such effect [494]. Melon seeds treated with Mn, Mo and Zn are said to produce fruit with an increased carotene content [529].

(g) *Environment*

Early reports indicated that oranges facing the sun have a higher carotenoid level in the peel than those facing away from the sun; this variation can be observed on opposite sides of the same specimen [466]. However, it has recently been demonstrated that peel from fruit on the north-facing side of a tree contains more carotenoid than those harvested from the south side [530].

Rose hips from bushes grown above 1200–1400 in Azerbaidzhan contain more carotenoids than those grown at a lower altitude [531].

(h) *Hormones*

Harvested tomatoes ripened in the presence of ethylene accumulate more total pigment than those ripened normally on the vine [532]. A similar effect is observed on the carotenoids, in particular  $\beta$ -citraurin [524, 533, 534], of the flavedo of citrus fruits; the effect was observed by post-harvest treatment with 10 p.p.m. ethylene. Ethephon and etherel, ethylene-releasing compounds, have a similar effect if applied preharvest [535, 536]. Neither  $\beta$ -naphthoxyacetic acid [537] nor  $\alpha$ -naphthylacetic acid [538] has any effect on pigment content of tomato fruit. 2,4-Dichlorophenylthiotriethylamine is similarly inert with citrus fruit [539]. Carotenoid synthesis in citrus fruit was inhibited by a cytokinin (benzyl adenine) (4.8) and by extremely low levels of gibberellic acid ( $GA_3$ ) (4.6) [538, 540, 541]; the effect can be observed both on and off the tree. In harvested fruit the effect is limited to the section of the peel directly exposed to the hormone, be it kinetin [542], 2,4-dichlorophenoxyacetic acid [543] or gibberellic acid [538]. There seems to be no translocation of the hormones. The effect of gibberellic acid is overcome by ethylene. It is possible that gibberellic acid is the controlling hormone *in vivo* but direct evidence for this is still awaited. Gibberellic acid also inhibits colour changes in tomatoes [544, 545] and bananas [546]. However, it stimulates carotenogenesis in grapes [547].

(i) *Pesticides*

Foschlor and Dipterex had no effect on the carotenoid content of tomato fruit [548].

(j) *General inhibitors*

CPTA inhibits formation of cyclic carotenoids, including capsanthin (1.30) and capsorubin (1.59); in various mutants of *Capsicum annuum* [407, 407A] there is a simultaneous accumulation of lycopene (1.11),  $\gamma$ -carotenes (1.38) and neurosporene (2.14). The chromoplasts from treated fruit lacked fibrils but this is probably not causally connected with impaired carotenogenesis. A dip (5 min) into ALAR (succinic acid 2,2-dimethyl hydrazide) (2500 p.p.m.) at 25° C has no effect on carotenoid formation in mango fruit; at 53° C, however, there is a stimulation of pigment synthesis, possibly because the climacteric is advanced [549]. A number of tertiary amines, general formula RCHN(C<sub>2</sub>H<sub>5</sub>)<sub>2</sub> inhibit the cyclization of lycopene in citrus fruit with the result that lycopene, rather than  $\beta$ -carotene, accumulates, sometimes in considerably increased amounts [550, 553]. In the case of aliphatic esters of 2-diethyl-aminoethanol, large amounts of lycopene accumulate soon after treatment, but later  $\beta$ -carotene is synthesized at the expense of lycopene, so that fruit are obtained which contain much  $\beta$ -carotene [554]. It is suggested that as the tissues slowly hydrolyse the esters the inhibitory effect on the cyclase enzyme disappears and the accumulated lycopene is converted into  $\beta$ -carotene.

(k) *Regreening*

If citrus fruit are left on the tree then they eventually will begin to synthesize chlorophylls [554] perhaps owing to the re-appearance of grana in chromoplasts, that is, as these organelles change back to chloroplasts [555].

## 5.1.6 TAXONOMIC SIGNIFICANCE OF CAROTENOIDS IN FRUIT

Attempts to assess the chemotaxonomic significance of carotenoid distribution in fruit have been made but are still hampered by lack of data [147]. It was thought that rubixanthin (4.9) in large amounts is characteristic of *Rosa* spp. but some species, particularly cultivars, seem to contain little if any of this pigment [147]. However, if an extract contains a substantial amount of rubixanthin, then it can be said with some confidence that it arose from the fruit of a *Rosa* sp. [235]. On the other hand, this conclusion cannot be extended to the Rosaceae family in general, because fruit of other genera such as *Cotoneaster*, *Crataegus*, *Pyracantha* and *Sorbus* do not accumulate rubixanthin. Wide divergences have also been observed amongst the Caprifoliaceae so far examined (Table 5.14). Marked variation can also be observed between different species of the same genus; for example *Pyracantha rogersiana* [235] and *P. coccinea* [274] produce insignificant amounts of 'plastid' carotenoids whilst *P. flava* synthesizes large amounts of derivatives of  $\beta$ -carotene, and *P. angustifolia* produces poly-*cis* carotenes [332]. Even



Table 5.14 Major carotenoid pigments in fruit of some Caprifoliaceae [235]

Pigment	Lonicera japonica	Lonicera periclymenum	Sambucus nigra	Viburnum opulus
Phytofluene (1.37)	+	?	—	+
$\beta$ -Carotene (1.13)	+	+	+	+
$\zeta$ -Carotene (1.47)	+	+	—	+
$\gamma$ -Carotene (1.38)	+	—	—	+
Lycopene (1.11)	+	+	—	+
Mutatochrome (1.44)	—	—	—	+
$\beta$ -Cryptoxanthin (1.60)	+	—	Trace	—
Zeaxanthin (1.18)	+	+	—	—
Lutein (1.74)	+	—	+	—
Flavoxanthin (4.3)	—	—	+	—
Chrysanthemaxanthin (4.2)	—	—	+	—
Auroxanthin (1.62)	+	+	—	—

capsanthin (1.30), long considered a pigment unique to peppers, has been found in some *Berberis* spp. (Table 5.8).

## 5.2 ROOTS

### (a) Distribution

The most important plants from the point of view of root carotenoids are the carrot and the sweet potato, although it should be recognized that the colour of some purple and yellow Asiatic carrots is due to anthocyanins [556]. Carrot root is of course the classic source of 'carotene' since 1831 when Wackenroder obtained it crystalline. 'Carotene' was separated into three isomers  $\alpha$ -,  $\beta$ - and  $\gamma$ -carotenes [557–560], following the development of column chromatography in the early 1930s [see [561] for further early references]. Since then traces of lycopene (1.11) [562],  $\delta$ -carotene (4.4) [562, 563],  $\zeta$ -carotene, (1.14) [560, 564], neurosporene (2.14), phytoene (1.36) and phytofluene (1.37) [563, 565–568] have been reported.

The xanthophyll fraction of commercial carrots is only 5–10% of the total pigments [569] but the percentage rises to 75–93% in yellow carrots [570] and to at least 95% in wild carrots [571, 572]. There is a differential distribution between xylem and phloem in commercial carrots; the xanthophylls are greater in the former where in Danvers Half Long and Yellow Belgian strains the percentage reaches 30–50% of the total [563]. There is also a quantitative differential distribution. The phloem has a 30% higher concentration than the xylem [563, 573, 574] and contains 80% of the root carotenoids [574, 575]. There is also a negative concentration gradient in moving from the inner to the outer layers of the phloem [543] and from the top to the tip of the root [563, 573–576].

The reported relative amounts of  $\alpha$ -carotene to  $\beta$ -carotene vary from 5–10% to 51% depending on the strain [562, 563, 577–579]; deep orange-red strains usually have relatively less  $\alpha$ -carotene than  $\beta$ -carotene [572]. The usual values for the carotene content of carrots are between 60 and 120  $\mu\text{g/g}$  (fresh wt.), although in some strains the value can be as high as 370  $\mu\text{g/g}$  (fresh wt.) [580]. European varieties appear to contain slightly more carotene than Asian varieties [581]; the red varieties such as Kin Toki, Tiny Tim and Big Boy have lycopene as their major pigment; it is accompanied by large amounts of  $\alpha$ -,  $\beta$ - and  $\gamma$ -carotenes [579, 582, 583].

$\beta$ -Carotene is also the main pigment present in sweet potatoes (*Ipomea batatas edulis*) and only traces of xanthophylls are found [584–588]. Very little carotene is present in the common potato and the amount does not vary greatly from the colourless to yellow varieties [564, 589–593]. The xanthophylls appear to be those of the chloroplast type [564, 590–593] and vary in amount from 0.2 to 2.6  $\mu\text{g/g}$  (fresh wt.) with most accumulating in the yellow varieties [564, 589, 591, 594].

Roots of swedes (*Brassica rutabaga*) are particularly interesting because they produce small amounts of poly-*cis* carotenes as well as some lycopene [595]. The apo-10'-carotenoid azafrin (1.79) has long been known to be present in the roots of *Escobedia linearis* and *E. scabrifolia* [596, 597] where it occurs together with small amounts of the corresponding aldehyde [598]. Violaxanthin (1.58) is the major pigment in the root cap of maize seedlings [598A]. It is probably formed by a light-independent epoxidation of the zeaxanthin present in the ungerminated seeds (Seeds 5.13(b)).

### (b) Localization

The pigments are located in carrots in chromoplasts, the structure of which has been described in detail [599–602]. The first particles seen are colourless vesicles (proplastids) about 1–2  $\mu\text{m}$  in diameter which do not begin to accumulate carotenoids until they are 3–5  $\mu\text{m}$  in diameter when they appear as yellow vesicles. About 16–28 days after germination carotenoid crystals appear as do starch grains. However, in older cells, more than 60 days after germination, two types of plastids are observed, those containing a starch-carotenoid complex and those containing carotenoid crystals which may be 40  $\mu\text{m}$  long [600]. In yellow and white mutants the chromoplasts only develop to the yellow and white vesicle stage respectively [600].

In the regions of the carrot which may be exposed to light chloroplasts are formed which can develop into chromoplasts [602]. In contrast chromoplasts in tissue culture will turn into chloroplasts under appropriate conditions [603].

In potatoes carotenoids are associated with all sub-cellular fractions but are concentrated in the membrane fraction and in the mitochondria [604].

(c) *Development*

As in the case of developing green tissues the carotene concentration in carrot roots increases with growth and becomes maximal about 100 days after sowing [541, 605–607]. During maturation the ratio of pigment in the phloem and xylem changes from 1.5:1 to 1.2:1 [606]. There is a negative correlation between carotene and sugar levels in the roots [607].

Stored carrots certainly maintain [574] and usually increase their pigment content, sometimes by up to 25%, until they begin to sprout [573, 608–610]. A high concentration of CO<sub>2</sub> (1.8–9.5%) increases formation on storage, as does a temperature of 0°C compared with 4°C particularly over a long term [611]. Similarly sweet potatoes increase their carotene content in storage by as much as 50% [612–614], the rate and increase depending on the variety [615].

(d) *Factors controlling synthesis*

*General.* In order to obtain autumn carrots with maximum carotene levels seeds must be sown before the end of May; summer sown carrots harvested in late winter and early spring have only one third the usual carotene content [616]. Soil moisture has no significant effect on the carotene concentration in carrot roots when measured on a dry weight basis [617]. However, on a fresh weight basis the carotene concentration is inversely proportional to soil moisture. This probably explains the claim that soil moisture influences the colour of carrots [618]. Carotene levels are higher in carrots grown on brown and loessial chernozem soil [619]. Thinning has no effect on pigment concentration [616] although there is an inverse relationship between size and concentration [580, 620]. Good carotene levels can be obtained by growing on an aeroponic conveyor [621].

In sweet potatoes cultivar differences are more important than production sites in controlling carotenoid levels [622], but increased levels have been observed in rainy years compared with drought years [623].

*Fertilizers.* Addition of N,P,K, (each 90 kg/ha) can improve the carotene levels in carrot roots by 50% [624, 625] although excessive application is of no value [625]. In pot experiments N (1 g/10 g soil) in the form of NH<sub>4</sub>NO<sub>3</sub> gave maximal carotene levels in carrots [626].

*Trace elements.* Boron [626], copper [626], manganese [627], cobalt [627] and molybdenum [627, 628] all have a positive effect on carotene formation in carrot roots.

*Herbicides and pesticides.* The effects of a number of agrochemicals on carotenoid production in carrots are recorded in Table 5.15.

*Pests.* Carrots infested with larvae of the carrot fly (*Psila rosae*) have slightly higher carotene levels than normal roots [616].

Table 5.15 Effects of herbicides and pesticides on carotene levels in carrots

<i>Agent</i>	<i>Effect</i>	<i>Ref.</i>
Afalon	None or reduction	629, 630
Ambien	Slight increase	631
Anthio	Increase	632
Busudin	Increase	632
CCC (2000 p.p.m.)	No effect	633
CIPC	Reduction	634
Fenitrothion (4 kg/ha)	Reduction	635
Gesagard	No effect	629
Lindane	Reduction	636
Linuron	{ Reduction	634
	{ Slight increase	637, 639
Metathion	Reduction	638
Nemagon	Increase	219, 640
Phthalophos	Increase	632
Propazine	Slight increase	631, 639
Prometryne	Slight increase	631, 639
Solan	Reduction	641
Telone	Increase	219, 640

*Hormones.* Although gibberellic acid (6–60 p.p.m.) stimulates carotenogenesis in carrot shoots it inhibits production in roots [642].

*Temperature.* Maximum pigment content in carrot roots is generally achieved in an environmental temperature of around 55–65°F (13–16°C) [603, 643–646].

#### (e) *Genetics*

Genetic studies on carrots have often been correlated with colour rather than carotenoid content, although quantitatively these could be expected to run together; however, there is evidence for the independent inheritance of phloem and xylem colour [572]. Only within the last few years have detailed studies on the inheritance of carotenoids in carrots been reported [572, 647]: five colour phenotypes, white, yellow, orange tinge, intermediate orange and orange were examined and it was found that white was dominant to all others and that the gene (*Y*) for the white phenotype almost completely inhibited carotene synthesis. A monogenic ratio was obtained for white and yellow crosses. Results with yellow and orange crosses revealed segregants for both dominance and non-dominance. The wide variation in carotene concentrations within the orange class suggested the presence of modifier genes. Studies with twenty eight varieties of carrot from different regions of the USSR showed that varietal differences were much more marked than differences caused by environmental changes [647]; for example, twice

repeated selection increased the carotene level by 32.6% [648, 649]. New hybrids of radish rich in carotene have been described during the last few years [650].

One wonders whether genes concerned with activities in the chromoplast may have effects on chloroplasts; carrot strains with white roots have no  $\alpha$ -carotene in their leaves although the  $\beta$ -carotene level is normal; strains with pigmented roots have a considerable amount of  $\alpha$ -carotene in the leaves [651].

#### (f) *Regreening*

Carrots will turn green on illumination, which is probably due to the chromoplasts changing into chloroplasts by forming a lamellar system and synthesizing chlorophylls [652].

#### (g) *Root tissue culture*

Green strains of carrot root cambial tissue culture synthesize small amounts of  $\alpha$ - and  $\beta$ -carotenes as well as xanthophylls; the orange strains contain much higher amounts of these carotenes but the xanthophyll fraction is increased only to a small extent [653]. (Table 5.16). Cultures from a single genetic source often differ in colour and can vary from red to orange (mainly carotenes)

Table 5.16 Carotenoid levels (mg/100 g fresh wt.) in two strains of carrot root tissue [653]

	<i>Green strain</i> (49 days old)	<i>Orange strain</i> (67 days old)	<i>Commercial</i> <i>carrots</i>
Total carotenoids	0.345	1.654	8.41
$\alpha$ -Carotene	—	0.254	0.652
$\beta$ -Carotene	0.165	0.319	7.595
Ratio xanthophylls/carotenes	1.08 : 1	0.18 : 1	0.02 : 1

to yellow (mainly xanthophylls) to white [654]. Four variant lines have been obtained by treatment of cultured carrot cells with the mutagen N-methyl-N'-nitro-N-nitrosoguanidine. They synthesized  $\beta$ -carotene and lycopene to varying extents, one, GD-3NR, producing an average 241  $\mu\text{g/g}$  dry wt. of  $\beta$ -carotene and 2344  $\mu\text{g/g}$  dry wt. of lycopene [655]. Another strain which synthesizes mainly lycopene has also been reported [553]. Triporic acid (Chapters 2 and 7) inhibits carotenogenesis in carrot tissue culture [656] as does kinetin [654], whilst 2,4-D stimulates synthesis [654, 657], probably by stimulating growth [654]. Carrot roots cultured *in vitro* do not synthesize carotenoids [658].

## 5.3. REFERENCES

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# [6]

## MOSESSES, LIVERWORTS AND SPORE-BEARING VASCULAR PLANTS

### 6.1 BRYOPHYTA—MOSESSES AND LIVERWORTS

After the pioneering work of Kohl at the turn of the century [1] which demonstrated the presence of carotenoids in *Funaria hygrometrica*, little of importance was published until the 1930s when carotenoids were noted in a number of moss gametophytes [2]. Many years again elapsed before typical higher plant chloroplast carotenoids, with the exception of neoxanthin (1.47), were found in the gametophytes and sporophytes of a number of mosses: *Entodon sedutrix*, *Mnium sylvaticum*, *Brachythecium acuminatum* and *Ditrichum vaginans* [3] as well as in *Fontinalis antipyretica* [4]. Later neoxanthin was found in *D. vaginans* and *E. sedutrix* and  $\beta$ -cryptoxanthin (1.60) was also noted in the former [5]. The usual chloroplast carotenoids were noted in *Sphaerocarpus donnellii* and no differences were noted between male and female plants; *cis*-isomers of  $\beta$ -carotene (1.13), not considered to be artifacts, were also present and  $\alpha$ -carotene (1.12) was absent [6]. The same pattern was also noted in *Lophocolea cuspidata* [4, 7], *L. bidentata* [8] and *Bryum ventricosum* [9].

In the case of *Sphaerocarpus donnellii* plants grown on soil produce more carotenoids than those grown on agar [6].

### 6.2 TRACOPHYTA—SPORE-BEARING VASCULAR PLANTS

A large number of plants examined by Strain and listed in Table 6.1 were found to contain the usual higher plant chloroplast carotenoids. *Selaginella* leaves turn red on 'bleaching' with the production of (probably) rhodoxanthin (4.10) [11–13]. The chloroplast structure in such leaves are intact but they contain, in addition, red osmiophilic droplets [14, 15] which presumably contain the accumulated rhodoxanthin.

Early work suggested that *Equisetum* spp. and *Adiantum* spp. [12] also contain rhodoxanthin and this has recently been confirmed for *Equisetum* by

Table 6.1 Spore-bearing vascular plants which have chloroplast carotenoids  
(Reference 10, except *Microsorium pustulatum* and *Pteridium aquilinum*)

<b>PSILOPSIDA</b>	<b>Ophioglossaceae</b>
<i>Psilotum complanatum</i> *	<i>Botrychium silai folium</i>
<i>P. nudum</i> *	<b>Polypodiaceae</b>
<b>LYCOPSIDA</b>	<i>Acrostichum</i> sp.*
<i>Lycopodium cernuum</i> *	<i>Adiantum</i> spp.* <sup>3</sup>
<i>Phylloglossum drummondii</i> <sup>2</sup>	<i>Asplenium lobulatum</i> *
<i>Splaginella arbuscula</i> *	<i>Blechnum indicum</i> *
<i>S. kraussiana</i> * <sup>2</sup>	<i>Cornuogramme pilosa</i> *
<i>S. wallacei</i>	<i>Elaphoglossum</i> spp.*
<b>SPHENOPSIDA</b>	<i>Microsorium pustulatum</i> *
<i>Equisetum arvense</i> *	<i>Nephrolepis exaltata</i> *
<i>E. telmateia</i> *	<i>Pelleae</i> sp.
<b>FILICOPSIDA</b>	<i>P. ternifolia</i> *
<b>Dickinsoniaceae</b>	<i>Pityrogramma calomelanos</i>
<i>Cibotium chamissoi</i>	<i>P. triangularis</i> *
<b>Gleicheniaceae</b>	<i>Platyterium bifurcatum</i> *
<i>Gleichenia dichotoma</i> *	<i>Polypodium</i> spp.*
<b>Hymenophyllaceae</b>	<i>Polystichum munitum</i> *
<i>Hymenophyllum recurvum</i> *	<i>Pteridium aquilinum</i>
<i>Trichomanes bauerianum</i> *	<i>Pteridium</i> spp.*
<b>Isoetacea</b>	<i>Pteris excelsa</i> *
<i>Isoetes bolanderi</i>	<i>Sadleria</i> sp.*
	<i>Sphenomeris chusana</i> *
	<i>Woodwardia finibriata</i> *

\* Contains  $\alpha$ -carotene in addition to  $\beta$ -carotene.

modern analytical methods [16] in spite of a report that male gametophytes of *E. arvense* and *E. hyemale* produce a pigment similar to but not identical to rhodoxanthin [17]. Bracken (*Pteridium aquilinum*) contains rather less  $\beta$ -carotene than do higher plants [18].

### 6.3 FUNCTION

The xanthophyll cycle (fully discussed in Chapter 3) also functions in ferns and bryophytes [19].

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## 7.1 NATURE AND DISTRIBUTION

## 7.1.1 CHLOROPHYTA

(a) *General distribution*

Under usual environmental and cultural conditions carotenoids are located in the chloroplasts of Chlorophyta. The pigments found in the class Chlorophyceae are those usually present in the chloroplasts of higher plants, that is  $\beta$ -carotene (1.13), lutein (1.74), violaxanthin (1.58) and neoxanthin (1.47); zeaxanthin (1.18), an occasional minor component of higher plant chloroplast carotenoids, is however widely distributed in the Chlorophyta [1, 2]. A series of *cis*-isomers of neoxanthin reported in *Chlorella ellipsoidea* may be artifacts [3]. Small amounts of  $\alpha$ -carotene (1.12) are frequently observed and the usual  $\alpha : \beta$  ratio is about 1 : 20; however *Chlamydomonas agloiformis* contains 'much  $\alpha$ -carotene' [4]. The xanthophyll:carotene ratio varies from about 1 : 3 in *Chlamydomonas reinhardtii* [5] to about 5.5 : 1 in *Nannochloris atomus* [6]. The Chlorophyceae known to contain 'higher plant' carotenoids are listed in Table 7.1.

As more and more algae are examined by modern methods an increasing number of exceptions to the general pattern outlined in Table 7.1 is being uncovered. Small amounts of loroxanthin (7.1), a pigment not known in higher plants, are present in a number of Chlorophyta (Table 7.2) from four different orders of the classes Chlorophyceae and Bryopsidophyceae [19]. This pigment is characterized by the fact that an in-chain methyl at C-19 has been oxidized to hydroxymethyl. Pyrenoxanthin from *Chlorella pyrenoidosa* ( $\equiv$  trollein) previously considered to be 3,20,3'-trihydroxy- $\alpha$ -carotene [20], is now known to be identical with loroxanthin [21, 22]. The pigments originally described as zeaxanthin and lutein in *Trentepohlia iolitus* (order Ulotrichales) have recently been characterized as 2-hydroxy- $\beta$ -carotene and 2-hydroxy- $\alpha$ -carotene, respectively [23]; they are accompanied by the cor-

Table 7.1 Chlorophyceae in which the major chloroplast pigments are the same as in higher plants (from Strain [4,7] unless otherwise stated)

<i>Chlorococcales</i>	<i>Ulotrichales</i>
Chlorococcaceae	Chaetophoraceae
<i>Chlorococcum</i> sp.*	<i>Chaetophora incrassata</i> *
<i>Chlorococcum humicola</i> (8)	<i>Draparnaldia glomerata</i> *
	<i>Draparnaldia</i> spp.*
Coelastraceae	<i>Microthamnium knetzingianum</i> <sup>c</sup> [12]
<i>Coelastrum proboscideum</i> *	<i>Pleurastrum pancicellulare</i> * [2]
<i>Trebouxia decolorans</i> <sup>a</sup> [9, 10]	<i>Stigeoclonium</i> spp.*
<i>T. humicola</i> <sup>a</sup> [9,10]	
	Protococcaceae
Coccomyxaceae	<i>Protococcus</i> spp.
<i>Coccomyxa elongata</i>	
<i>Coccomyxa</i> spp. [2]	Ulotrichaceae
<i>C. viridis</i>	<i>Hormidium</i> spp.*
<i>C. simplex</i>	<i>Stichococcus subtilis</i> *
	<i>Ulothraix</i> spp.
Hydrodictyaceae	UVALES
<i>Hydrodictyon</i> spp. [2]	<i>Enteromorpha clathrata</i>
<i>Pediastrum boryanum</i> [2]	<i>E. compressa</i>
	<i>E. intestinalis</i>
Oocystaceae	<i>E. spp.</i> *
<i>Ankistrodesmus braunii</i> * [11]	<i>E. tubulosa</i>
<i>A. falcatus</i>	<i>Monostroma</i> spp.*
<i>Ankistrodesmus</i> spp.* [2]	<i>Ulva fasciata</i> *
<i>Chlorella pyrenoidosa</i> <sup>a,b</sup>	<i>U. lactuca</i> *
<i>C. variegata</i> [8]	<i>U. latissima</i>
<i>C. vulgaris</i> *	<i>U. linza</i> *
	<i>U. reticulata</i> *
Scenedesmaceae	<i>U. spp.</i>
<i>Scenedesmus bifulgatus</i> *	<i>D. salina</i> *
<i>S. brasiliensis</i> *	<i>D. spp.</i> * [2,15]
<i>S. obliquus</i> *	<i>D. tertiolecta</i> * [16,17]
VOLVOCALES	
	Volvocaceae
Chlamydomonadaceae	<i>Eudorina elegans</i> [2]
<i>Chlamydomonas agloeiformis</i> *	CHAETOPHORALES
<i>C. reinhardtii</i> <sup>b</sup> [5,13]	<i>Fritschiella tuberosa</i> [18]
<i>Chlorogonium elongatum</i> * [2]	<i>Pleurastrum pancicellulare</i> <sup>d</sup> [2]
<i>Haematococcus pluvialis</i> * [2]	
Pedinomonadaceae	
<i>Pedinomonas tuberculata</i> * [2]	
Polyblepharidaceae	
<i>Dunaliella primolecta</i> [14]	

#### Notes

<sup>a</sup> The free living *T. humicola* has the same pigments as *T. decolorans*, the phycobiont of the lichen *Xanthoria parietina*.

<sup>b</sup> Also loroxanthin (7.1).

<sup>c</sup> Unexpectedly produces siphonaxanthin, Section 7.1.1.

<sup>d</sup> Also lutein epoxide.

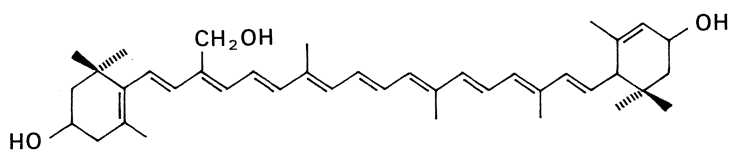
\* Indicates presence of  $\alpha$ -carotene as well as  $\beta$ -carotene.

Table 7.2 Green algae in which loroxanthin has been detected

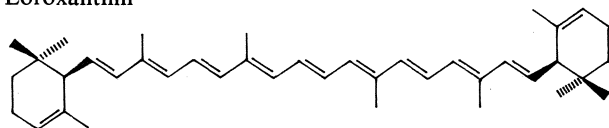
Algae	Order	Class	Ref.
<i>Chlamydomonas reinhardtii</i>	Volvocales	Chlorophyceae	21
<i>Chlorella vulgaris</i>	Chlorococcales	Chlorophyceae	19
<i>Cladophora trichotoma</i>	Cladophorales	Bryopsidophyceae	19
<i>C. ovoidea</i>	Cladophorales	Bryopsidophyceae	19
<i>Scenedesmus obliquus</i>	Chlorococcales	Chlorophyceae	19
<i>Ulva rigida</i>	Ulvales	Chlorophyceae	19

responding monoepoxides and by 2,2'-dihydroxy- $\beta$ -carotene [24]. This was the first report of naturally occurring carotenoids hydroxylated at position 2; such pigments have now been found in blue-green algae but they have the opposite chirality at C-2 (*R*) from that in the *Trentepohlia* pigments (2*S*) (Section 7.1.10(a)).

In the Bryopsidophyceae siphonaxanthin (2.19) exists as a characteristic pigment of the Siphonales [25–28]. It appears to be a constant constituent of members of the Derbesidales, Codiales and Cauleropales, and is sporadically distributed amongst the Cladophorales and Siphonocladales but it has not yet been found in any Acrosiphonales, Sphaeropicales and Dasyclades [29]. An esterified form of siphonaxanthin, siphonein [26], accompanies the free pigment in almost all the algae examined except some Siphonocladales; conversely siphonein, but not siphonaxanthin, is found in some Dichotomosiphonales [29]. The distribution of the two pigments is summarized in Table 7.3. 'Siphonein' is likely to represent a group of pigments differing in



(7.1) Loroxanthin



(7.2)  $\epsilon$ -Carotene

the fatty acid with which siphonaxanthin is esterified. For example in *Caulerpa prolifera* the esterifying acid is lauric acid [30], in *Codium fragilis* [25] it is not lauric acid but possibly a mixture of fatty acids is involved [27]. All the siphonaxanthin-containing algae so far examined except one are also characterized by a higher content of  $\alpha$ -carotene than  $\beta$ -carotene; the exception is *Dichotomosiphon tuberosus*, the only fresh-water specimen in the group

Table 7.3 Distribution of siphonaxanthin and siphonein in green algae  
(Class: Bryopsidophyceae)

(From Kleinig, [29] except where otherwise stated)

A = siphonaxanthin; B = siphonein; - = absence of both pigments. Otherwise usual chloroplast pigments present

<i>Cladophorales</i>		<i>Sphaeropleales</i>	
<i>Chaetomorpha aerea</i> [4, 7, 21]	-	<i>Sphaeroplea annulina</i>	-
<i>C. antennina</i> [4, 7, 21]	-	<i>S. cambrica</i>	-
<i>C. linum</i>	-		-
<i>C. melagonium</i>	-	Dasycladales	-
<i>C. sp.</i>	-	<i>Acetabularia clavata</i> [4, 7, 31]	-
<i>Cladophora crispata</i> [4, 7, 31]	-	<i>A. crenulata</i> [4, 17, 31]	-
<i>C. glomerata</i> [4, 7, 31]	-	<i>A. mediterranea</i> <sup>b</sup>	-
<i>C. fascicularis</i> [4, 7, 31]	-	<i>A. mobii</i> [4, 7, 31]	-
<i>C. graminea</i> [4, 7, 31]	-	<i>A. wettsteinii</i>	-
<i>C. membranacea</i> (4, 7, 31)	-	<i>Acicularia (Acetabularia) schenckii</i>	-
<i>C. rupestris</i>	-	<i>Batophora oerstedii</i>	-
<i>C. cf. prolifera</i>	A	<i>Bornetella sphaerica</i> [4, 7, 31]	-
<i>C. cf. lehmanniana</i>	A	<i>Cymopolia barbata</i>	-
<i>C. sp.</i> [4, 7, 31]	-	<i>Dasycladus clavaeformis</i>	-
<i>C. sp.</i>	A,B	<i>Neomeris annulata</i> [4, 7, 31]	-
<i>C. trichotoma</i> <sup>a</sup> [4, 7, 19, 31]	-		
<i>Rhizoclonium implexum</i> [4, 7, 31]	-	Siphonocladales	-
<i>R. tortuosum</i>	-	<i>Anadyomene stellata</i>	-
<i>Spongomorpha coalita</i> [4, 7, 31]	-	<i>Blastophysa rhizopus</i>	A
Acrosiphoniales	-	<i>Boodlea coacta</i>	A
<i>Acrosiphonia arcta</i>	-	<i>B. kaeneana</i> [4, 7, 31]	-
<i>A. sonderi</i>	-	<i>Cladophoropsis herpestica</i> [16]	-
Derbesiales		<i>C. zollingeri</i>	-
<i>Derbesia lamourouxii</i> [4, 7, 31]	A,B	<i>Dictyosphaeria cavernosa</i> [4, 7, 31]	-
<i>D. tenuissima</i>	A,B	<i>D. favulosa</i> [4, 7, 31]	-
<i>D. spp.</i>	A,B	<i>D. versluysii</i> [4, 7, 31]	-
<i>D. vaucheriaeformis</i> [4, 7, 31]	A,B	<i>Microdictyon setchellianum</i>	
<i>Halicystis ovalis</i> [4, 7, 31]	A,B	[4, 7, 31]	-
		<i>Struvea spp.</i> [4, 7, 31]	
Codiales		<i>Valonia fastigiata</i> [4, 7, 31]	
<i>Avrainvillea nigricans</i>	A,B	<i>V. macrophysa</i>	A
<i>A. rawsoni</i>	A,B	<i>V. utricularis</i>	A
<i>Bryopsis corticulans</i> [4, 7, 31]	A,B	<i>Valoniopsis pachynema</i> [16]	-
<i>B. hypnoides</i> [4, 7, 31]	A,B		
<i>B. muscosa</i>	A,B	Caulerpales	
<i>B. spp.</i>	A,B	<i>Caulerpa cupressoides</i> [4, 7, 31]	A,B
<i>Chlorodesmis comosa</i> [4, 7, 31]	A,B	<i>C. distichophylla</i> [4, 7, 31]	A,B
<i>Codium bursa</i>	A,B		
<i>C. coronatum</i> [4, 7, 31]	A,B	<i>C. filiformis</i> <sup>c</sup> [4, 7, 31]	A,B
<i>C. dimorphum</i>	A,B	<i>C. lentillifera</i> [4, 7, 31]	A,B
<i>C. duthieae</i> [4, 7, 31]	A,B	<i>C. prolifera</i>	A,B
<i>C. elongatum</i>	A,B	<i>C. racemosa</i> [4, 7, 31]	A,B
<i>C. fragile</i> [25]	A,B	<i>C. serrulata</i> [4, 7, 31]	A,B

<i>Cladophorales</i>		<i>Sphaeropleales</i>	
<i>C. lucasii</i> [4, 7, 31]	A,B	<i>C. sertularioides</i> [4, 7, 31]	A,B
<i>C. muelleri</i> [4, 7, 31]	A,B	<i>Halimeda discoidea</i> [4, 7, 31]	A,B
		<i>H. opuntia</i> [4, 7, 31]	A,B
<i>C. spongiosum</i>	A,B	<i>H. tuna</i> [4, 7, 31]	A,B
<i>C. tomentosum</i>	A,B	<i>Udotea flabellum</i> [4, 7, 31]	A,B
<i>Penicillus capitatus</i> [4, 7, 31]	A,B	<i>U. petiolata</i>	A,B
		Dichotomosiphonales	
		<i>Dichotomosiphon</i> spp.	B
		<i>D. tuberosus</i>	B

#### Notes

<sup>a</sup>Also loroxanthin

<sup>b</sup>An earlier report [32] of astaxanthin and lutein 5,6-epoxide was not confirmed in a later investigation [33].

<sup>c</sup>There are contradictory results with this organism.

examined [31]. Both siphonaxanthin and siphonein exist in the alga *Ostreobium* which exists symbiotically with the brain coral *Favia* [6].

Many Prasinophyceae contain the usual higher plant carotenoids (Table 7.4) but some e.g. *Asteromonas propulsa*, produce in addition siphonaxanthin and siphonein; others, such as *Pterosperma* spp. and *Heteromastix* spp., are characterized by the absence of lutein as well as the presence of siphonaxanthin and siphonein [33, 34]. It thus appears that siphonaxanthin and siphonein occur significantly only in the classes Bryopsidophyceae and Prasinophyceae, but that a member of the Chaetophoraceae, *Microthamnion knetzingianum*, synthesizes siphonaxanthin [12], which is unusual. Two Chlorophyceae symbionts of *Lubomiska baicalensis*, *Draparnaldia baicalensis* and *Zoochlorella* spp. synthesize somewhat different pigments. The former

Table 7.4 The Prasinophyceae which contain normal chlorophycean carotenoids [33, 34]

<i>Haematococcus</i> spp.	<i>P. subcordiformis</i>
<i>Heteromastix rotunda</i>	<i>P. striata</i>
<i>H.</i> spp.	<i>Prasinocladus lubricus</i>
<i>Mesostigma viride</i>	<i>P. marinus</i>
<i>Monomastix minuta</i>	<i>P. sp.</i>
<i>Pedinomonas minor</i> <sup>a</sup>	<i>Pyramimonas obovata</i>
<i>Platymonas chuii</i>	<i>P. urceolata</i>
<i>P. tetrathele</i>	<i>P. grossii</i>
	<i>Spermatozopsis exsultans</i>

#### Note

<sup>a</sup>Also synthesizes  $\gamma$ -carotene and lycopene in significant amounts.

produces  $\beta$ -carotene,  $\epsilon$ -carotene (7.2), lutein (1.74), lutein 5,6-epoxide and neoxanthin (1.47) with lutein esters, rather surprisingly, predominating [35]; the latter synthesizes  $\beta$ -carotene,  $\beta$ -cryptoxanthin (1.60), antheraxanthin (1.24), lutein and zeaxanthin [35].

The polymer, sporopollenin, which may arise by polymerization of carotenoids, has been detected in the trilaminar wall layers of *Chlorella fusca* var. *vacuolata* [36].

Two members of the order Charales, *Chara fragilis* [2] and *C. foetida* [4], synthesize the expected chloroplast carotenoids with small amounts of  $\gamma$ -carotene (1.38); in addition traces of antheraxanthin (1.24) are present in the former and lycopene (1.11) in the latter.

A representative set of values for the quantitative distribution of carotenoids in green algae is given in Table 7.5.

### (b) Localization

As in higher plants carotenoids normally exist in the chloroplasts of the Chlorophyta although they are said to be present in the cell wall of *Scenedesmus obliquus* [37]. There can be differential distribution within the chloroplast, for in the phototactic organism *Chlamydomonas reinhardtii* the eye spot is located inside the chloroplast. The eye spots have been isolated by differential centrifugation and were found to contain only  $\beta$ -carotene [38].

The fragmentation of *Chlorella* chloroplasts into particles with photosystems I and II activity revealed a differential distribution of carotenoids in the particles similar to that observed in particles prepared from higher plant chloroplasts (Chapter 4); more xanthophylls than carotenes in the photosystem II particles and the reverse in photosystem I particles [39]. The light-harvesting chlorophyll a/b protein complex from *Chlamydomonas reinhardtii* contains qualitatively all the carotenoids present in the chloroplasts [40].

A yellow, chlorophyll-free, water-soluble carotenoid protein has been obtained from *Scenedesmus obliquus*. It has a molecular weight of 140 000 and the carotenoid:protein ratio was approximately 1:1. The absorption maxima were located at 424, 449 and 479 nm which represented a shift to red compared with the maxima of the free pigment, violaxanthin, in acetone (420, 441 and 470 nm). The protein-bound violaxanthin represents only about 1% of the total violaxanthin in the alga [40A]. The nature of the protein-pigment binding is not known.

Carotenoids are found outside the chloroplasts either in fruiting areas of certain colonial species or in the cytoplasm under certain unfavourable cultural conditions. The gametes of the fruiting areas of *Ulva lobata* [24] and *U. lactuca* [41] have a carotene content some 4–6 times greater than in the non-fruiting areas and this is mainly due to the specific synthesis of  $\gamma$ -carotene (1.38). This pigment and lycopene (1.11) also accumulate in the antheridia of *Chara ceratophylla* and *Nitella syncarpa* [42].

Table 7.5 Quantitative distribution of carotenoids in typical members of the green algae [2] (mg/g dry wt.)

PIGMENT	EUCHLOROPHYCEAE			ULOTHRICOPHYCEAE			ZYGO-PHYCEAE	CHARO-PHYCEAE
	<i>Volvocales</i> <i>Dunaliella</i> <i>spp.</i>	<i>Chlorococcales</i> <i>Ankistrodesmus</i> <i>spp.</i>	<i>Chaetophorales</i> <i>Pleurastrum</i> <i>paucicellulare</i>	<i>Odegoniales</i> <i>Oedogonium</i> <i>cardiacum</i>	<i>Siphonales</i> <i>Protosiphon</i> <i>botryoides</i>	<i>Zygnematales</i> <i>Zygnema</i> <i>circumcarinatum</i>	<i>Charales</i> <i>Chara</i> <i>foetida</i>	
<i>α</i> -Carotene (1.12)	0.02	0.29	0.03	0.33	0.36	—	—	
<i>β</i> -Carotene (1.13)	0.88	0.52	0.41	0.69	0.82	0.64	0.26	
Violaxanthin (1.58)	0.42	0.34	0.19	0.41	0.61	0.54	1.03	
Lutein (1.74)	2.44	1.09	1.01	1.62	2.68	1.39	0.25	
Antheraxanthin (1.24)	0.42	0.08	0.03	0.06	0.09	0.23	0.03	
Neoxanthin (1.47)	0.64	0.35	0.36	0.48	0.57	0.44	0.08	
Zeaxanthin (1.18)	1.02	0.43	0.16	0.19	0.09	0.32	0.05	
Others	—	0.93 <sup>a</sup>	0.30 <sup>b</sup>	0.39 <sup>c</sup>	—	—	0.06 <sup>c</sup>	
TOTAL	5.84	3.99	2.49	4.17	3.22	3.56	1.76	

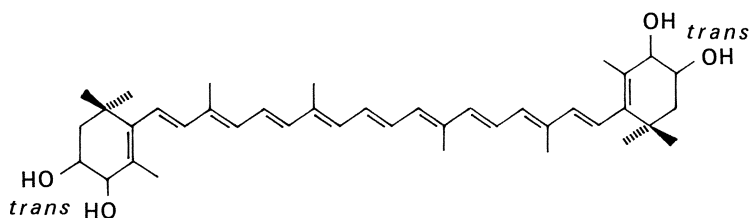
Notes

<sup>a</sup> Canthaxanthin (1.40),  $\beta$ -Cryptoxanthin (1.60), (?) trihydroxy- $\alpha$ -carotene.

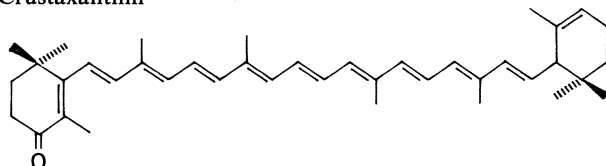
<sup>b</sup> Lutein-5,6-epoxide, (?) trihydroxy- $\alpha$ -carotene.

<sup>c</sup>  $\gamma$ -Carotene (1.38).

The extra-plastidic pigments which are found under adverse nutritional conditions, usually nitrogen deficiency, are normally  $\beta$ -carotene and/or its keto derivatives, echinenone, (1.22), hydroxyechinenone, canthaxanthin (1.40) and astaxanthin (1.68); more rarely crustaxanthin (7.3) and phenicopterone (7.4) are found in smaller amounts. The known distribution of extra plastidic carotenoids is given in Table 7.6. The localization of these carotenoids appears to vary with species; they occur in intracytoplasmic deposits which have no limiting membranes in *Protosiphon botryoides* [71] and in lipid vacuoles in *Ankistrodesmus braunii* [72]. The globules have been isolated from *P. botryoides* by gradient centrifugation on sucrose [73]. In *Haematococcus pluvialis* the pigments have been described as occurring in plastoglobuli in



(7.3) Crustaxanthin



(7.4) Phenicopterone

aplanospores [55, 74] outside any organelle or vesicle [65], and in 'perinuclear cytoplasm characterized by a network of ribosome-coated endoplasmic reticulum segments', free ribosomes, dictyosomes in active stages of vesicle formation, and mitochondria [72]. A number of mutants of *Chlorella pyrenoidosa* are known which have lost the ability to synthesize canthaxanthin (1.40) on a nitrogen-poor medium; however they frequently revert to the wild type and regain their ability to synthesize secondary carotenoids [75]. A green alga, similar to *C. fusca* isolated from the lichen *Haematomma ventosum* behaves similarly to other Chlorococcales in synthesizing the usual pigments under normal nutritional conditions and keto carotenoids under nitrogen starvation [76].

During the accumulation of secondary carotenoids in *Ankistrodesmus braunii* and *Chlorella fusca* var. *rubescens* there is no significant loss of the chloroplast carotenoids although the organelles may themselves undergo considerable structural changes resulting particularly in a decrease in the number of thylakoids present [77]. However other reports suggest that in *A. falcatus*, *Chlorella zofingiensis*, *Chlorococcum wimmerii* and *Chromo-*



Table 7.6 Green algae which produce extra-plastidic carotenoids under unfavourable conditions

Alga	Pigments	Ref.
<i>Acetabularia mediterranea</i>	?	43, 44
<i>Ankistrodesmus</i> spp. <sup>a</sup>	1, 2, 3	45
<i>Brachiomonas simplex</i>	3	46
<i>Chlamydomonas nivalis</i>	1, [2], 3	47–50
<i>Chlorella fusca</i> ( <i>pyrenoidosa</i> )	1, 2	51
<i>C. fusca</i> var. <i>rubescens</i> <sup>b</sup>	1, 2, 3	52
<i>C. zofingiensis</i>	1, 2, 3	50, 53, 54
<i>Chlorococcum infusionum</i>	1, 2, 3	47
<i>C. multinucleatum</i>	1, 2, 3	47
<i>C. olefaciens</i>	1, [2], 3	47
<i>C. wimmerii</i>	1, 2, 3, 4	47, 55
<i>Chromochloris cinnabarina</i>	?	50
<i>Coelastrum proboscideum</i> var. <i>dilatatum</i>	1, 2, (3)	47
<i>Crucigenia apiculata</i>	1, 2, (3)	47, 51
<i>Dictyococcus cinnabarinus</i>	1, 2, 5 <sup>c</sup>	47, 54, 56, 57
<i>Frittschiella tuberosa</i>	1, 2, 3, 14 <sup>f</sup>	57A
<i>Gymnozya moniliformis</i>	(1)	58
<i>Haematococcus droebakensis</i>	1, 2, 3	47
<i>H. lacustris</i> <sup>d</sup>	1, 2, 3, 10	59
<i>H. pluvialis</i>	1, 3, 4, 11, 12	47, 50, 60–65
<i>Hydrodictyon reticulatum</i>	1, 3	47, 53
<i>Nannochloris atomus</i>	3	32
<i>Protosiphon botryoides</i> <sup>e, f</sup>	1, 2, 3, 6 <sup>d</sup> , 7 <sup>d</sup> , 8 <sup>d</sup> , 9, 13	66, 67
<i>Scenedesmus brasiliensis</i>	1	51
<i>Scenedesmus</i> spp. <sup>g</sup>	1, 2, 3	45, 50, 51
<i>Scotiella</i> spp.	1, 2, 3	47
<i>Sphaeroplea</i>	?	68
<i>Spongiochloris typica</i>	?	69
<i>Trentepohlia aurea</i>	10	70

## Key

- |  |   |
|--|---|
| 1. Echinenone (1.22)                         | 8. 3-Hydroxy-4-4'-diketo- $\beta$ -carotene     |
| 2. Canthaxanthin (1.40)                      | (= phoenicoxanthin, adonirubin)                 |
| 3. Astaxanthin (1.68)                        | 9. 3-Hydroxy-4,3',4'-triketo- $\beta$ -carotene |
| 4. 3,4,4'-trihydroxy- $\beta$ -carotene      | 10. $\beta$ -Carotene (1.13)                    |
| 5. 3,4-diketo- $\beta$ -carotene             | 11. Crustaxanthin (7.3)                         |
| 6. 4'-Hydroxyechinenone                      | 12. Phoenicopteron (7.4)                        |
| 7. 4-Hydroxy,3',4'-diketo- $\beta$ -carotene | 13. 3,4-dihydroxy-4'-keto- $\beta$ -carotene    |
|  | 14. Frittschiellaxanthin <sup>h</sup> (7.6)     |

## Notes

<sup>a</sup> Present in 25 strains.<sup>b</sup> Previously named *Halochlorella rubescens*.<sup>c</sup> Pigment(s) only tentatively identified.<sup>d</sup> Not present in all strains.<sup>e</sup> Five strains examined.<sup>f</sup> The hydroxylated pigments are esterified.<sup>g</sup> Present in 27 strains.<sup>h</sup> Previously considered to be  $\alpha$ -doradexanthin (7.5) [11] which is an epimer of frittschiellaxanthin [57A].

*chloris cinnbarina* there is a concomitant loss of plastid carotenoids during formation of secondary carotenoids [50].

The colour of the 'blood-rain' and 'blood snow' caused naturally by the sudden bloom of the aplanospores of *Haematococcus pluvialis* and *Chlamydomonas nivalis* is due to keto-carotenoids which accumulate under conditions of nitrogen deficiency [78].

#### (c) *Pigment changes during cell cycle*

A start has been made on this important problem by studying the changes in *Chlamydomonas reinhardtii* on a 12 h light, 4 h dark cycle. During the light period all pigments increased at a similar rate, but lutein (1.74) and violaxanthin (1.58) synthesis precedes that of  $\beta$ -carotene (1.13) neoxanthin (1.47) and linoxanthin (7.1). A marked drop in all pigments was observed after nine hours which corresponds to the known loss of RNA and break-up of the nucleus which occur at this time [79]. The relatively small changes noted in the dark probably require further detailed study. Variations have also been reported during the cell cycle of *Scenedesmus cicutus* [80].

#### (d) *Mutants*

Various mutants of green algae have been isolated and examined in detail. The polyenes formed in a number of these are listed in Table 7.7. In addition to those listed the pigments in a large number of additional *Chlorella* mutants have been examined [81, 82].

A series of mutants of *Chlamydomonas reinhardtii* and *Scenedesmus* spp. which have impaired ability to photosynthesize produce essentially the normal complement of carotenoids, although there are some qualitative variations [13, 83]. One mutant PGI of *S. obliquus* produces different pigments according to whether it is grown in light or darkness (see Section 7.2.3(b)).

### 7.1.2 RHODOPHYTA

The hydrocarbons  $\alpha$ -carotene (1.12) and  $\beta$ -carotene (1.13) and their corresponding xanthophylls lutein (1.74) and zeaxanthin (1.18) are the usual carotenoid pigments of red algae [4, 7, 87] which thus generally exhibit a very simple qualitative picture. There are, however, considerable quantitative differences; for example zeaxanthin frequently preponderates over lutein, a situation rarely if ever observed in green algae, and in *Polysiphonia collinsii* [4], *Porphyridium aerugineum* [88], *P. cruentum* [89] and *Asterocytis ramosa* [88] lutein appears to be absent.  $\alpha$ -Carotene frequently preponderates over  $\beta$ -carotene particularly in the Delesseriaceae [4, 7]; it is also frequently absent but there is only one report of a red alga, *Phycodrys sinuosa* [90], from which  $\beta$ -carotene is absent. The known distribution of carotenoids in red algae is given in Table 7.8.

Table 7.7 Carotenoid composition of some mutants of *Chlorella* and *Scenedesmus*

Mutant	Polyenes	Ref.
<i>Chlamydomonas reinhardtii</i> pale-green	5 and 9 at concentrations 200–500 times lower than normal: no xanthophylls	5
<i>Chlorella vulgaris</i> 5/871 5/518 9a 5/520	1 only 1, 2, 3–no xanthophylls 1, 2, 3, 4–no 5 but normal xanthophylls In light: normal pigments In darkness: 1, 2, 3, 4, 6, 7–no 5 or xanthophylls	278, 279
<i>Chlorella pyrenoidosa</i> G34 G41 G44	1 only Normal pigments 1, 3 + 9 unidentified xanthophylls	
<i>Scenedesmus obliquus</i> IN IQ C61 C55 II IK PGI	{ none – although chlorophyll present 1 only 1 only 1, 2, 3, 4 <sup>a</sup> , 8 1, 2, 3, 4, 8 In light: normal pigments In darkness: 1, 2, 3, 4, 8 (5 + 9) <sup>b</sup> ; traces of normal xanthophylls	

## Key

1. Phytoene	(1.36)	6. Proneurosporene	
2. Phytofluene	(1.37)	7. Prolycopene	(1.55)
3. $\zeta$ -Carotene	(1.14)	8. 3-Hydroxy- $\beta$ -zeacarotene	
4. $\beta$ -Zearcarotene	(2.13)	9. $\alpha$ -Carotene	(1.12)
5. $\beta$ -Carotene	(1.13)		

## Notes

<sup>a</sup> Traces.<sup>b</sup> Traces of a mixture detected.

The comparatively simple qualitative distribution of carotenoids in red algae,  $\alpha$ - and  $\beta$ -carotenes, lutein and zeaxanthin, although very widespread, is not universal. The epoxyxanthophyll antheraxanthin (1.24) was reported as the main pigment in two red algae, *Acanthophora spicifera* and *Gracilaria lichenoides*, from Hawaii [91]; however, it was not detected in *G. sjoesledtii* from California [4] or in *G. edulis* from Australia. Violaxanthin (1.58) was found in *Halosaccion glandiforma* [4] and a report of the possible presence of neoxanthin (1.47) in one specimen of *Nemalion multifidum* [87] needs con-

Table 7.8 Carotenoids in red algae

	Pigments	Ref.
<b>BANGIALES</b>		
<i>Asterocystis ramosa</i>	1, 2	4
<i>Bangia fuscopurpurea</i>	1, 2, 3	97
<i>Erythrotrichia carnea</i>	1, 2	4
<i>Porphyra naiadum</i>	1, 2, 3	4
<i>P. perforata</i>	1, 2, 3, 5	4
<b>PORPHYRIDIALES</b>		
<i>Porphyridium aeruginum</i>	1, 2, 4	88, 89
<i>P. cruentum</i>	1, 2, 4	4, 89
<i>Rhodorus marinus</i>	1, 2	7
<b>NEMALIALES</b>		
<i>Asparagopsis armata</i>	1, 2, 3, 5	7
<i>A. taxiformis</i>	1, 2, 3, 5	7
<i>Batrachospermum</i> spp.	1, 2, 3, 4, 5	7, 89
<i>Bonnemaïsonia hamifera</i>	1, 2, 3, 5	97
<i>Cumagloia andersonii</i>	1, 2, 3, 5	4
<i>Galaxaura</i> spp.	1, 2, 3, 5	7
<i>G. umbellata</i>	1, 2, 3, 5	7
<i>Gloiophloeia confusa</i>	1, 2, 3, 5	7
<i>Nemalion helminthoides (multifidum)</i>	1, 3, 5	7, 97
<i>Rhodochorton rothii</i>	1, 2, 3, 5	7
<b>CERAMIALES</b>		
<i>Amansia dietrichiana</i>	1, 2, 3, 5	7
<i>Antithamnion plumula</i>	1, 3	87, 98
<i>Botryoglossum farlowianum</i>	1, 2, 3, 5	4
<i>Callithamnion californianum</i>	1, 2, 3, 5	4
<i>C. pikeanum</i>	1, 2, 3, 5	4
<i>Callophyllis marginifructa</i>	1, 2, 3, 5	4
<i>Centroceras clavatum</i>	1, 2, 35	4
<i>C. davulata</i>	1, 2, 3, 5	7
<i>Ceramium eatonianum</i>	1, 2, 3, 5	4
<i>C. rubrum</i>	1, 2, 3, 5, 6	97
<i>Chondria</i> spp.	1, 2, 3	7
<i>Cryptopleura lobulifera</i>	1, 2, 3, 5	4
<i>C. violacea</i>	1, 2, 3, 5	4
<i>Dilsea carnosa (edulis)</i>	1, 3	98
<i>Griffithsia pacifica</i>	1, 2, 3, 5	4
<i>Griffithsia</i> spp.	1, 2, 3, 5	7
<i>Hymenena flabelligera</i>	1, 2, 3, 5	4
<i>H. kylinii</i>	1, 2, 3, 5	4
<i>H. multiloba</i>	1, 2, 3, 5	4
<i>Laurencia heteroclada</i>	1, 2, 3	7
<i>L. nidifica</i>	1, 2, 3	4
<i>L. obtusa</i>	1, 2, 3, (5)	7
<i>L. pacifica</i>	1, 2, 3	4
<i>L. rigida</i>	1, 2, 3	7
<i>Laurencia</i> spp.	1, 2, 3	7
<i>L. spectabilis</i>	1, 2, 3	4
<i>Lophosiphonia villum</i>	1, 2, 3, 5	4
<i>Martensia</i> spp.	1, 2, 3, 5	7

	Pigments	Ref.
<i>Microcladia borealis</i>	1, 2, 3, 5	4
<i>M. coulteri</i>	1, 2, 3, 5	4
<i>Nienburgia andersoniana</i>	1, 2, 3, 5	4
<i>Odothalia floccosa</i>	1, 2, 3, 5	4
<i>Polyneura latissima</i>	1, 2, 3, 5	4
<i>Polysiphonia aquamara</i>	1, 2, 3	4
<i>P. brodiaei</i>	1, 2, 4	97
<i>P. californica</i>	1, 2, 3	4
<i>P. collinsii</i>	1, 2, 3	4
<i>P. fastigiata</i>	1, 2	87
<i>P. urceolata</i>	1, 2, 4	97
<i>Pterocladia capillacea</i>	1, 2, 4, 5	99
<i>P. lucida</i>	1, 2, 35	7
<i>Pterosiphonia baileyi</i>	1, 2, 3	4
<i>P. bipinnata</i>	1, 2, 3	4
<i>P. dendroidea</i>	1, 2, 3, 5	4
<i>Ptilota densa</i>	1, 2, 3, 5	4
<i>Rhomomela larix</i>	1, 2, 3	4
<i>Ricardia saccata</i>	1, 23	4
<i>Spermothamnion snyderae</i>	1, 2, 35	4
<i>Spyridia filamentosa</i>	1, 2, 3, 5	7
<b>CRYPTONEMIALES</b>		
<i>Bossiella corymbifera</i>	1, 2, 3, 5	4
<i>B. orbigniana</i>	1, 2, 3, 5	4
<i>B. plumosa</i>	1, 2, 3, 5	4
<i>Calliarthron cheilosporioides</i>	1, 2, 3	4
<i>C. setchelliae</i>	1, 2, 3	4
<i>Corallina chilensis</i>	1, 2, 3, (5)	4
<i>C. gracilis</i>	1, 2, 3, 5	4
<i>C. rosea</i>	1, 2, 3, 5	7
<i>Cryptosiphonia woodii</i>	1, 2, 3, 5	4
<i>Endocladia muricata</i>	1, 2, 3, 5	4
<i>Farlowia compressa</i>	3, 5	4
<i>F. mollis</i>	1, 2, 3, 5	4
<i>Grateloupia californica</i>	1, 2, 3, 5	4
<i>G. filicina</i>	1, 2, 3, 5	4, 97
<i>G. proteus</i>	1, 2, 3, 5	97
<i>G. setchellii</i>	1, 2, 3, 5	4
<i>Lithophyllum neofarlowii</i>	1, 3	(4)
<i>Prionitis andersonii</i>	1, 2, 3, 5	4
<i>P. australis</i>	1, 2, 3, 5	4
<i>P. lanceolata</i>	1, 2, 3, 5	4
<b>GELIDIALES</b>		
<i>Gelidium coulteri</i>	1, 2, 3, 5	4
<i>Pterocladia capillacea</i>	1, 2, 3, 5	7
<i>P. lucida</i>	1, 2, 3, 5	7

## Key

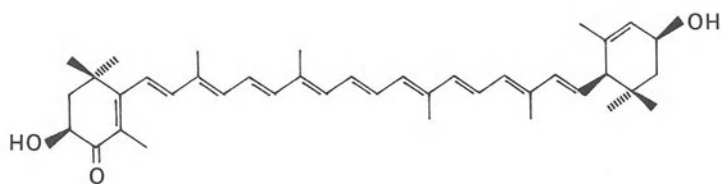
- |                      |        |                           |        |
|----------------------|--------|---------------------------|--------|
| 1. $\beta$ -Carotene | (1.13) | 4. $\beta$ -Cryptoxanthin | (1.60) |
| 2. Zeaxanthin        | (1.18) | 5. $\alpha$ -Carotene     | (1.12) |
| 3. Lutein            | (1.74) |                           |        |

firmation.  $\beta$ -cryptoxanthin (1.60) was present in the two Hawaiian algae just mentioned [91] and in traces in *Porphyridium aerugineum*, *P. cruentum* and *Bactrachospermum* [89], and  $\alpha$ -cryptoxanthin (4.1) appears in *Lenormandia prolifera* [92]. *Cyanidium caldarium* is taxonomically difficult to place. At the moment it appears to be located in the Rhodophyta probably as a new family Cyanidiaceae within the order Porphyridiales [93]. Its carotenoid spectrum:  $\beta$ -carotene (1.13), zeaxanthin (1.18) and lutein (1.74), would support this conclusion [94]. The occasional reports in earlier papers of fucoxanthin (1.57) in red algae, in particular *Callithamnion pikeanum* [26], *Ceramium rubrum* [95] and *Polysiphonia nigrescens* [96], are probably due to the presence of contaminating diatoms in the samples.

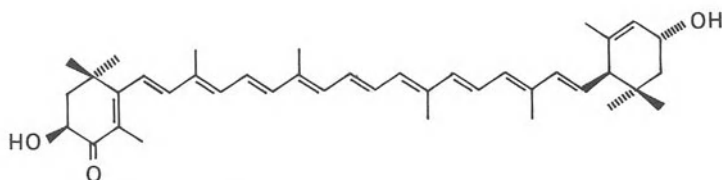
### 7.1.3 PYRROPHYTA

The chemistry of the carotenoids of the Pyrrophyta has only recently been put on a firm basis. The characteristic pigment of the phylum, peridinin (1.71), was first isolated in 1927 from *Peridinium cinctum* [96] but its structure was only elucidated [100] in 1971 and full details given in 1976 [101, 102]. Its similarity to fucoxanthin (1.57), the characteristic carotenoid of the Phaeophyceae, is clear. In peridinin the oxidation of an in-chain methyl group, first noted in green algae as a hydroxymethyl group [loroxanthin (7.1), siphonaxanthin (2.19)], has been carried further to a carboxylic group which then forms a lactone. However, peridinin differs fundamentally from fucoxanthin in being a *nor*-carotenoid, in that it has lost three in-chain carbon atoms (formally considered as C-12, 13 and 20 of fucoxanthin). This is a unique structural feature in naturally occurring carotenoids. There are a number of reports of fucoxanthin in Pyrrophyta, for example *Gymnodinium veneficum* [103], *Peridinium foliaceum* [104, 105], *P. balticum* [106], a *P. sp.* [105] and an *Exuvilla sp.*, in this case a *cis*-isomer, neofucoxanthin, was isolated [106]. The presence of fucoxanthin, rather than peridinin, in *Peridinium foliaceum* and *P. balticum* is particularly interesting because they both contain two nuclei, one dinokaryotic and one characteristically eukaryotic [107, 108]. *P. foliaceum* contains oil droplets which accumulate a very uncharacteristic mixture of polyenes, phytoene (1.36), phytofluene (1.37),  $\zeta$ -carotene (1.14),  $\beta$ -zeaxanthin (2.13),  $\beta$ -carotene (1.13) and  $\gamma$ -carotene (1.38) [109]. It is suggested that these pigments are produced by an apochlorotic host dinoflagellate whilst  $\beta$ -carotene (1.13), fucoxanthin (1.57) and diadinoxanthin (2.28) are produced by an endosymbiont Chrysophyte [108, 110]. It is significant that the apochlorotic dinoflagellate *Cryptocodinium cohnii* contains  $\beta$ -carotene and  $\gamma$ -carotene as the major carotenoid components [112].

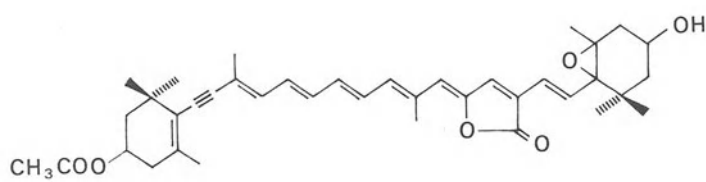
Peridinin represents between 38 and 84% of the total carotenoids present in the Pyrrophyta which synthesize it [113]. Accompanying peridinin in most Pyrrophyta are  $\beta$ -carotene (1.13), diatoxanthin (2.27), pyroxanthin (7.7), dinoxanthin (7.8), diadinoxanthin (2.28), pyroxanthinol (7.9) and peridinol



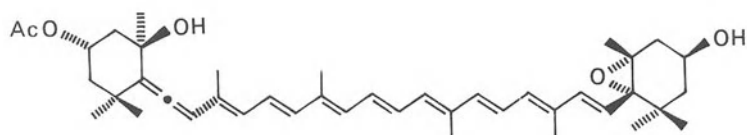
(7.5)  $\alpha$ -Doradexanthin



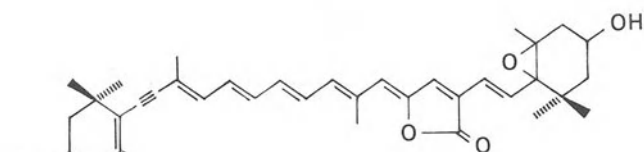
(7.6) Fritschiellaxanthin



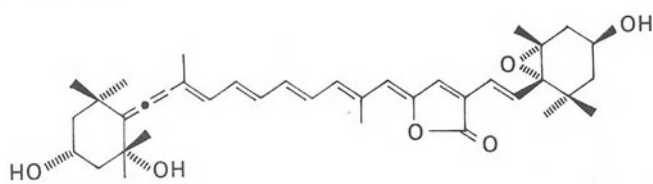
(7.7) Pyrroxanthin



(7.8) Dinoxanthin



(7.9) Pyrroxanthinol



(7.10) Peridininol

(7.10) [113, 114]. The characteristics of these pigments include the presence of acetylenic linkages (diadinoxanthin, pyroxanthin) and the presence of acetoxy groups (peridinin, pyroxanthin, dinoxanthin). The demonstration for the first time of the presence of deacetylated pigments such as peridininol and pyroxanthin is important: no such derivative of fucoxanthin has yet been reported. Rather unexpectedly astaxanthin (1.68) has been found in a *Glenodinium* species [113]. The general distribution of pigments in the Pyrrophyta is given in Table 7.9.

Table 7.9 Qualitative distribution of carotenoids in the Pyrrophyta

<i>Alga</i>	<i>Pigment</i>	<i>Ref.</i>
<b>DESMOPHYCEAE</b>		
Prorocentrales		
<i>Exuviaella cassubica</i>	1, 2, 3, 4	105
<i>Exuviaella</i> spp.	1, 2, 3, 7 <sup>a</sup>	106, 115
<i>Prorocentrum micans</i>	1, 2, 3, 4	116, 117
<b>DINOPHYCEAE</b>		
Gymnodiniales		
Gymnodiniaceae		
<i>Amphidinium carteri</i>	1, 2, 3, 4, 6, (?), 8	105, 113, 118, 119
<i>A. corpulentum</i>	1, 2, 3, 4	105
<i>A. hoeffleri</i>	1, 2, 3, 4	105
<i>A. klebsi</i>	1, 2, 3, 4	104, 105, 120
<i>A. rhyncocephalum</i>	1, 2, 3, 4	105
<i>A.</i> spp.	1, 2, 3, 4	121, 128, 129
<i>Gymnodinium nelsoni</i>	1 <sup>b</sup> , 2, 3, 4, (?6), (?7), 8	113
<i>G. punctatum</i>	1, 2, 3, 4	105
<i>G. simplex</i>	1, 2, 3, 4	105
<i>G. splendens</i>	1 <sup>b</sup> , 2, 3, 4, (?8)	105, 113
<i>G.</i> spp.	1, 2, 3, 4	105, 120, 122
<i>G. veneficum</i>	1, 4, 5	103
<i>Gyrodinium galatheanum</i>	1, 4, 5, 7, 12	299
Glenodiniaceae		
<i>Glenodinium</i> spp. <sup>c,d</sup>	1 <sup>b</sup> , 2, 3, 4, 6, 7, 8, 10	105, 113
<i>Gyrodinium dorsum</i> <sup>d</sup>	1 <sup>b</sup> , 2, 3, 4, 6, 8, 9	105, 113
<i>G. resplendens</i>	1 <sup>b</sup> , 2, 3, 4, 6	105, 114
Peridiniaceae		
<i>Aureodinium pigmentosum</i>	3	111, 115
<i>Cachonina niei</i> <sup>d</sup>	1, 2, 3, 4	105, 123
<i>Ensiculifera loeblichii</i> <sup>d</sup>	1, 2, 3, 4	105
<i>Peridinium balticum</i>	1, 4, 5, 7, 11	105, 106
<i>P. cinctum</i>	1, 2, 3, 4	123
<i>P. foliaceum</i> <sup>c,d</sup>	1, 4, 5, 11	104, 105, 109
<i>P. trochoideum</i>	1, 2, 3, 4	105, 111, 115
<i>Woloszynskia micra</i>	5	111
<i>W.</i> spp.	5	111
Gonyaulacaceae		
<i>Gonyaulax polyedra</i>	1, 2, 3, 4	105, 124



<i>Alga</i>	<i>Pigment</i>	<i>Ref.</i>
ZOOXANTHELLAE <sup>e</sup>		
<i>Anemonia sulcata</i> (sea anemone)	3 <sup>f</sup>	119
<i>Pocillipora</i> spp. (coral) <sup>g</sup>	1, 2, 3, 4	121
<i>Tridaena crocea</i> (clam) <sup>g</sup>	1, 2, 3, 4	121
Protoceratiaceae		
<i>Protoceratium reticulatum</i> <sup>d</sup>	1, 2, 3, 4	105
Phytodinaceae		
<i>Pyrocystis lunula</i> <sup>d</sup>	1, 2, 3, 4	105

#### Key

1. $\beta$ -Carotene	(1.13)	7. Diatoxanthin	(2.27)
2. Dinoxanthin	(7.8)	8. Peridininol	(7.10)
3. Peridinin	(1.77)	9. Pyrroxanthinol	(7.9)
4. Diadinoxanthin	(2.28)	10. Astaxanthin	(1.68)
5. Fucoxanthin	(1.57)	11. $\gamma$ -Carotene	(1.38)
6. Pyrroxanthin	(7.7)	12. 19'-Hexanoyloxyfucoxanthin	

#### Notes

<sup>a</sup> Both fucoxanthin and peridinin reported in different investigations.

<sup>b</sup>  $\beta$ -Carotene identified.

<sup>c</sup> See p. 222 for further discussion.

<sup>d</sup> Also unidentified pigments.

<sup>e</sup> The pigments are in the symbiotic Dinophyceae living in the animals listed.

<sup>f</sup> First isolated as sulcatoxanthin [119].

<sup>g</sup> The isolated organism is *Gymnodinium microadriaticum* [105].

The symbiotic zooxanthellae which exist in marine animals contain the same carotenoids as the free-living animals: indeed peridinin was first isolated from the sea anemone *Anemonia sulcata* and named sulcatoxanthin [116].

#### 7.1.4 EUGLENOPHYTA

Carotenoids in *Euglena* chloroplasts are similar to green leaf carotenoids in consisting of  $\beta$ -carotene (1.13), zeaxanthin (1.18) and neoxanthin (1.47) [125, 126] but differ significantly in that the main xanthophyll is the acetylenic pigment diadinoxanthin (2.28) [127] and not antheraxanthin (1.24) [126] or lutein (1.74) [125] as previously suggested. Diatoxanthin (2.27) [128, 129] and heteroxanthin (7.16) [124] have also recently been reported, as has depoxyneoxanthin (= ? trollein) [130]. The same pigments are found in *Trachelomonas hispida* var. *coronata* [128]. Traces of the ketocarotenoids echinenone (1.22), 3-hydroxyechinenone, canthaxanthin (1.40), euglenanone and astaxanthin (1.68) are usually also present [126, 128, 131]. The structure of euglenanone is still obscure [132] but it may be identical to canthaxanthin.

These keto-carotenoids are probably located in the eyespots of *Euglena* spp. because in chlorotic strains of *E. gracilis* in which the chloroplasts but not the eyespots have been removed, echinenone remains but no plastid carotenoids can be detected [131]. However, one report exists that lutein (? diatoxanthin) is the eyespot pigment [133]. The availability of pure eyespots may eventually resolve some of these discrepancies [134].

Table 7.10 summarizes the carotenoid pigments present in strains of *Euglena* which have become permanently mutated to achlorotic forms following exposure to certain antibiotics and other metabolic inhibitors, or to excessive hydrostatic pressure. The concentration of streptomycin which bleaches dividing organisms is the same as that required to inhibit chloroplast development (i.e. carotenoid synthesis) in illuminated non-dividing dark-grown cells [137]. *Euglena* spp. will also grow heterotrophically in the dark when they do not produce chloroplasts but plastids; carotenoids are synthe-

Table 7.10 Carotenoids in non-photosynthetic *Euglena* mutants [131, 135, 136]

Strain	Mutagenic agent	Carotenoids	
		Carotenes	Xanthophylls
PBZG1	Pyribenzamine	Phytofluene, $\beta$ -carotene, $\zeta$ -carotene-like	—
PB2G2		Phytoene, phytofluene $\beta$ -carotene, $\zeta$ -carotene-like	—
PB2G3			
SML1	Streptomycin	Phytoene, phytofluene, $\beta$ -carotene, $\zeta$ -carotene-like	absent or present
SMP		Phytofluene $\beta$ -carotene, $\zeta$ -carotene-like	only in traces
SMG			
HBG	High temperature (30°C)	Phytoene, phytofluene, $\beta$ -carotene, $\zeta$ -carotene-like	Echinenone, zeaxanthin, diadinoxanthin in traces
PR1	High pressure	Phytoene, phytofluene, $\zeta$ -carotene, $\beta$ -zeacarotene	—
PR2			
PR3		$\beta$ -carotene	
PR4		No polyenes detectable	—

sized but in reduced amounts [138]. In mutants which synthesize polyenes more saturated than  $\beta$ -carotene and lycopene, such as phytoene, as the only components, plastids are present in dark-grown cultures; however they are absent from mutants grown in the dark and which do not produce such polyenes [139]. Inhibition of carotenoids occurs in both resting and growing cells of *Euglena* on addition of the herbicide SAN 9789; in growing cells the original pigment is diluted out as the cells divide. If the herbicide is removed there is no change in the carotene level of the resting cells indicating that there is no significant turnover of the pigment [140].

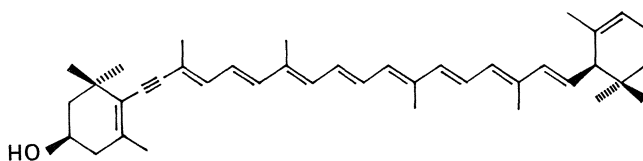
## 7.1.5 CHLOROMONADOPHYTA (RHAPHIDOPHYTA)

Only two members of this phylum, which consists of one order, the Chloromondales, have been examined up to the time of writing. *Vacuolaria virescens* and *Gonyostomum semen* contain  $\beta$ -carotene (1.13), antheraxanthin (1.24) (? = diadinoxanthin), lutein 5,6-epoxide (?), and a hydroxy lutein epoxide-like pigment (= ?heteroxanthin) [141]. Further work is obviously needed although at first sight the pattern seems similar to that noted in the Xanthophyceae (Section 7.1.7).

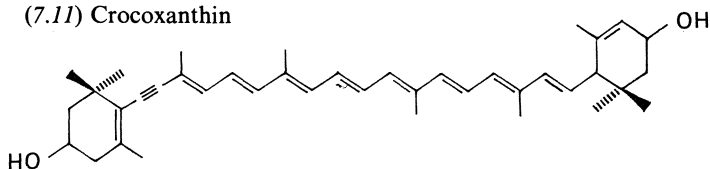
## 7.1.6 CRYPTOPHYTA

(a) *Cryptomonadales*

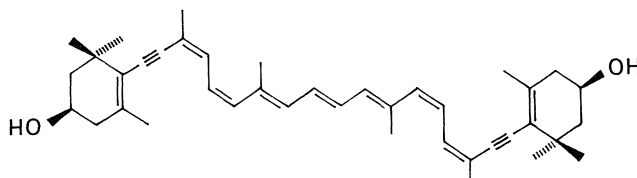
The algae so far investigated in this order include three *Cryptomonas* spp. (now known as *Chroomonas salina*), *Cryptomonas ovata* and *Rhodomonas* spp. from the family Cryptomonadaceae, and one, *Hemiselmis viridis*, from the family Senniaceae. They all are similar in that  $\alpha$ -carotene (1.12) predominates over  $\beta$ -carotene (1.13) [87, 142] and that the major xanthophyll is the acetylenic alloxanthin (1.46) [143–145]. Other acetylenes, crocoxanthin (7.11) (in *H. viridis* and *Rhodomonas* sp.) and monadoxanthin (7.12) (in *Chroomonas salina*) are present in smaller amounts [142]. The rather rare carotene  $\epsilon$ -carotene (7.2) is found in *Cryptomonas ovata* [142]. It is interesting that bleached autolysed cultures of *Chroomonas salina* contain 9,9'-dicis alloxan-



(7.11) Crocoxanthin



(7.12) Monadoxanthin



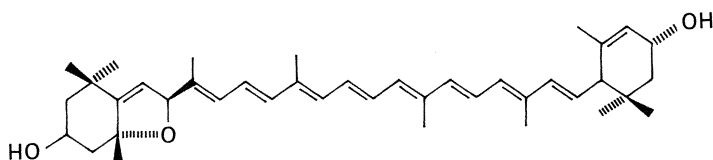
(7.13) 9,9'-Dicisalloxanthin

thin (7.13) which is a very stable *cis*-isomer produced artifactually by light [146]. A recent report rather surprisingly suggests that *Rhodomonas baltica* contains  $\alpha$ -carotene (1.12), diatoxanthin (2.27), diadinoxanthin (2.28) and fucoxanthin (1.57) [147].

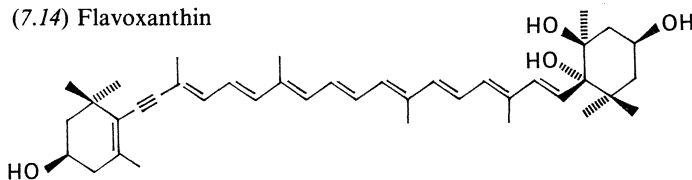
### 7.1.7 CHRYSOPHYTA

#### (a) *Xanthophyceae* (*Heterokontae*)

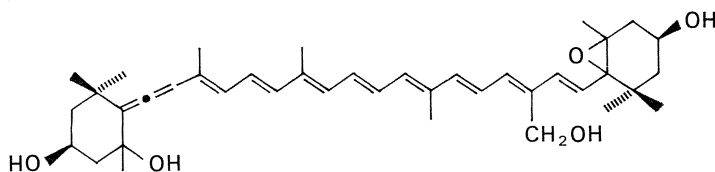
Xanthophylls were first reported in *Chlorobotrys stellata* [148] in 1930 and the main pigment, originally thought to be flavoxanthin (7.14) [149], was also reported in *Botrydium granulatum* [150]. Later investigations revealed the presence of three to four unidentified xanthophylls [4, 64, 151–153]. Three constant components have been identified as diadinoxanthin (2.28), diatoxanthin (2.27) and heteroxanthin (7.15) [89, 145, 154–156A]. Vaucherixanthin (7.16) in the form of a partial ester is frequently but not always present; neoxanthin (1.47) has also been reported once [157]. The known distribution of carotenoids in heterokonts is given in Table 7.11.



(7.14) Flavoxanthin



(7.15) Heteroxanthin



(7.16) Vaucherixanthin

#### (b) *Chrysophyceae*

The genus *Ochromonas* has been examined in most detail and *O. danica* [94] and *O. stipitata* [2] contain  $\beta$ -carotene (1.13), violaxanthin (1.58), fucoxanthin (1.57), antheraxanthin (1.24) and zeaxanthin (1.18) with  $\beta$ -cryptoxanthin

Table 7.11 Carotenoids in Xanthophyceae

<i>Alga</i>	<i>Pigments</i>	<i>Ref.</i>
<b>VAUCHERIALES</b>		
Botydiaceae		
<i>Botrydium becherianum</i>	1, 6 <sup>a</sup>	150
<i>B. granulatum</i>	1, 2, 3, 4, 5, 6, 7, 8, 9	89, 155
Vaucheriaceae		
<i>Vaucheria dichotoma</i>	1 <sup>b</sup>	158
<i>V. germinata</i>	1 <sup>b</sup>	158
<i>V. sessilis</i>	1, 4, 6, 7, 8	145, 155
<i>V. spp.</i>	4, 6, 8	154
<i>V. tenestris</i>	6, 7	155
Centritracteaceae		
<i>Bumilleriopsis brevis</i>	1, 6 <sup>a</sup>	150
<i>B. filiformis</i>	1, 2, 3, 4, 5, 6, 7, 8, 10	89
<b>TRIBONEMETALES</b>		
Heterotrichaceae		
<i>Bumilleria exilis</i>	1, 6	150
<i>B. sicula</i>	1, 2, 3, 4, 5, 6, 7, 8, 10	89
<i>Hetrothrix debilis</i>	1, 2, 3, 4, 5, 6, 7, 8, 10	4, 89, 150
Tribonemataceae		
<i>Tribonaema aequale</i>	1, 2, 3, 4, 5, 6, 7, 8, 10, 11	89, 150, 153, 156, 159
<i>T. bombycinum</i>	6, 7, 8	4, 159
<i>T. minus</i>	6, 7, 8	4, 159
Heterodeudraceae		
<i>Heterococcus caespitosus</i>	1, 2, 3, 4, 5, 6, 7, 10	89
<i>H. fuorensis</i>	1, 6 <sup>a</sup>	150
<b>MISCHOCOCCALES</b>		
(Heterococcales)		
<i>Mischococcus sphaerocephalus</i>	1, 4, 12	153
<i>Ophiocytium majus</i>	4, 6, 8	150, 160

**Key**

- |   |        |                                   |        |
|---|--------|-----------------------------------|--------|
| 1. $\beta$ -Carotene                          | (1.13) | 7. Diatoxanthin                   | (2.27) |
| 2. $\beta$ -Cryptoxanthin 5,6,5',6'-diepoxide |        | 8. Heteroxanthin                  | (7.15) |
| 3. $\beta$ -Cryptoxanthin 5,6-epoxide         |        | 9. Zeaxanthin                     | (1.18) |
| 4. Vaucherixanthin-ester                      | (7.16) | 10. $\beta$ -Carotene-5,6-epoxide |        |
| 5. Neoxanthin                                 | (1.47) | 11. Unknown carotene              |        |
| 6. Diadinoxanthin                             | (2.28) | 12. Antheraxanthin                | (1.24) |

**Notes**<sup>a</sup>Originally reported as antheraxanthin.<sup>b</sup>Plus 3 unknown xanthophylls.

(1.60) and  $\beta$ -cryptoxanthin-5,6-epoxide present in small amounts in *O. stipitata*. Another member of this class, *Pseudopedinella* sp., also synthesizes fucoxanthin [161].

### (c) *Haptophyceae*

The carotenoids in a number of Haptophyceae are summarized in Table 7.12. It is clear that fucoxanthin (1.57) is present in all species except *Emeliana huxleyi* in which it is replaced by 19-*n*-hexanoyloxyfucoxanthin. In all cases

Table 7.12 Carotenoids in Haptophyceae

Organism	Pigments	Ref.
<i>Chrysochromulina ericina</i>	1	161
<i>Dictaeria inomata</i>	1	161
<i>Emeliana huxleyi</i> <sup>a</sup>	2, 3	145, 162–164
<i>Hymenomonas carterae</i>	1, 3, 4, 5, 9, 10	145, 163
<i>Isochrysis galbana</i>	1, 3, 4, 5, 11, 7 <sup>c</sup> , 8	2, 103, 120, 122, 161, 164,
<i>Isochrysis</i> spp.	138	166
<i>Pavlova gyrans</i>	1	161
<i>Pavlova lutheri</i> <sup>b</sup>	1, 3, 4, 5, 9, 11, 12 <sup>c</sup> , 13	2, 164
<i>Pavlova</i> spp. (NEP)	1, 3, 4, 5, 6	145, 164
<i>Phaeocystis pouchetii</i>	1	101
<i>Prymnesium parvum</i>	1, 3, 4, 5, 7, 11	94, 164
<i>Sphaleromantis</i> spp.	1	122
<i>Syracosphaera carterae</i>	1	2

#### Key

- |   |  |
|---|--|
| 1. Fucoxanthin (1.57)                           | 8. Echinenone (1.22)                               |
| 2. 19'- <i>n</i> -Hexanoyloxyfucoxanthin (2.28) | 9. $\beta$ -Carotene-5,6-epoxide (1.60)            |
| 3. Diadinoxanthin (1.13)                        | 10. $\beta$ -Cryptoxanthin (1.60)                  |
| 4. $\beta$ -Carotene (2.27)                     | 11. $\beta$ -Cryptoxanthin-5,6,5',6'-diepoxide (?) |
| 5. Diatoxanthin (1.12)                          | 12. Canthaxanthin (1.40)                           |
| 6. Fucoxanthinol (see Chapter 1)                | 13. Dincoxanthin (7.8)                             |

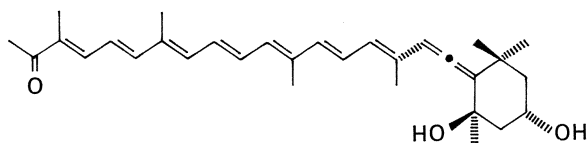
#### Notes

<sup>a</sup> Previously *Coccolithus huxleyi*.

<sup>b</sup> Previously *Monochrysis lutheri*.

<sup>c</sup> Traces.

fucoxanthin or its derivative is the major carotenoid component. The earlier report of 19-hexanoyloxyparacentrone (7.17) and diatoxanthin (2.27) in *E. huxleyi* [145] was not confirmed in the later experiments [114]. The appearance of the 4-keto carotenoids echinenone (1.22) and canthaxanthin (1.40) in two members of this class, *Isochrysis galbana* and *Pavlova lutheri*, respectively [165] is unexpected.



(7.17) Paracentrone

(d) *Eustigmatophyceae*

A number of members of this recently defined class [166] have been examined, many at a time when they were included in the Xanthophyceae. The known distribution is given in Table 7.13.

Table 7.13 Carotenoids in Eustigmatophyceae

Alga	Pigments	Ref.
<i>Botrydiopsis alpina</i>	1, 2, 3, 4, 5, 6	89, 150
<i>Chlorobotrys regularis</i>	3 <sup>a</sup> , (?4), 8	160
<i>Pleurochloris commutata</i>	1, 3, 4, 7, 8, 9	89, 153
<i>Pleurochloris magna</i>	1, 3 <sup>c</sup> , 4 <sup>b</sup> , 8 <sup>c</sup> , (?11)	145, 153
<i>Polyedriella helvetica</i>	1, 4, 8	4, 153
<i>Vischeria</i> spp.	1, 9 <sup>d</sup>	87, 150
<i>Vischeria stellata</i>	5, 6, 10	4
GBS-Sticho <sup>e</sup>	1, 3, 8 <sup>c</sup> , 11	153
Clone Tunis <sup>e</sup>	1, 3 <sup>a,c</sup> , 8 <sup>c</sup> , 11	153

## Key

1. $\beta$ -Carotene	(1.13)	7. Zeaxanthin	(1.18)
2. $\beta$ -Cryptoxanthin-5,6-epoxide		8. Violaxanthin	(1.58)
3. Vaucherixanthin	(7.16)	9. Antheraxanthin	(1.24)
4. Neoxanthin	(1.47)	10. Heteroxanthin	(7.15)
5. Diadinoxanthin	(2.28)	11. Canthaxanthin	(1.40)
6. Diatoxanthin	(2.27)		

## Notes

<sup>a</sup> Free and esterified.

<sup>b</sup> Not reported in later investigations.

<sup>c</sup> Furanoid derivatives also present.

<sup>d</sup> Probably diadinoxanthin also present.

<sup>e</sup> Unnamed algae – probably Eustigmatophytes.

(e) *Bacillariophyceae* (diatoms)

The diatoms so far examined in pure culture (Table 7.14) have a carotenoid distribution rather similar to that found in the Phaeophyta (see next section) in that the major pigments are  $\beta$ -carotene (1.13), diatoxanthin (2.27), diadinoxanthin (2.28) and fucoxanthin (1.57) [4, 7]. The earlier reports of the presence of lutein (1.74) and zeaxanthin (1.18) may have been misidentifications for diadinoxanthin and diatoxanthin, respectively. The rare  $\epsilon$ -carotene (7.2) is present *Nitzschia closterium* [123] and *Navicula pelliculosa* [2]. In

*Phaeodactylum tricornutum* (previously named *Nitzschia closterium* f. *minutissima*) the level of fucoxanthin drops as the culture ages whilst that of diadinoxanthin increases: the  $\beta$ -carotene level remains constant throughout [167].

Table 7.14 Diatoms which contain the four carotenoids indicated in the text

Diatom	Ref.
<i>Cymbella cymbiformis</i> <sup>a</sup>	169
<i>Fragilaria sublinearis</i>	121
<i>Isthmia nervosa</i>	123
<i>Melosira</i> sp.	2
<i>Navicula ostrearia</i>	170
<i>Navicula pelliculosa</i>	2
<i>Navicula torquatum</i>	123
<i>Nitzschia closterium</i> (= <i>Phaeodactylum tricornutum</i> ) <sup>b</sup>	2, 122, 123, 168
<i>Nitzschia closterium</i> f. <i>minutissima</i>	7, 123
<i>Nitzschia dissipata</i>	171
<i>Nitzschia palea</i>	123
<i>Stephanopyxis turris</i>	123
<i>Thalassiosira gravida</i>	123

Notes

<sup>a</sup> Also possibly lutein.

<sup>b</sup> The furanoid isomer of diadinoxanthin appears in old cultures.

### 7.1.8 PHAEOPHYTA

#### (a) General distribution

A wide-ranging investigation [172] revealed that the major pigments of the Phaeophyta are  $\beta$ -carotene (1.13), violaxanthin (1.58) and fucoxanthin (1.57), with fucoxanthin predominating. The yearly production of algal carotenoids in the oceans of the world is  $1.2 \times 10^7$  tons or  $360 \text{ mg/m}^{-2}$ , of this some  $6.8 \times 10^6$  tons are fucoxanthin which makes it the most abundant carotenoid in the world [173]. No  $\alpha$ -carotene (1.12) has been observed in species of the Phaeophyta obtained, for example, from such widely dispersed locations as Norway [172] and Australia [7]. Some genera from Norway contained zeaxanthin (1.18) but it was difficult to decide whether or not it was a *post mortem* artefact, as suggested earlier. The naturally occurring fucoxanthin is the all-*trans* form and traces of *cis*-forms occasionally reported may have been artefacts [4, 123]. Traces of diatoxanthin (2.27) and diadinoxanthin (2.28) are observed in some Phaeophyta and they are not considered to be the result of contamination with diatoms.

The absorption spectrum *in vivo* of brown algae in the region 500–560 nm is different from that of the extracted pigments. This has been considered to be due to the existence of a ‘fucoxanthin-protein complex’. Recently such a



Table 7.15 Phaeophyta which have been examined for carotenoids  
(excluding those listed in Table 7.16)

Alga	Ref.	Alga	Ref.
<i>Alaria esculenta</i>	172	<i>Leathesia difformis</i>	172
<i>Ascophyllum nodosum</i>	172–175	<i>Macrocystis integrifolia</i>	4
<i>Bachelotia fulvescens</i>	4	<i>Myriogloria (Haplogloia)</i>	7
<i>Colpomenia sinuosa</i>	4, 7	<i>sciurus</i>	
<i>Cystophyllum muricatum</i>	7	<i>Nemacystus decipiens</i>	7
<i>Cystoseira osmundacea</i>	4	<i>Padina</i> spp.	4, 7
<i>Desmarestia aculeata</i>	172, 176	<i>Pelvetia canaliculata</i>	172, 173
<i>Dictyopteris</i>	7	<i>Pelvetiopsis limitata</i>	4
<i>acrostichoides</i>		<i>Petalonia fascia</i>	7, 172
<i>Dictyosiphon</i>	172	<i>Petrospongium rugosum</i>	4, 7
<i>foeniculaceus</i>		<i>Phaeosaccion collinsii</i>	175
<i>Dictyota</i> spp.	4, 7, 174, 176	<i>Phyllospora comosa</i>	7
<i>Ecklonia radiata</i>	7	<i>Pocockiella nigrescens</i>	7
<i>Egregia menziesii</i>	4	<i>Postelsia palmaeformis</i>	4
<i>Fucus</i> spp.	4, 7, 172–177	<i>Pterygophora californica</i>	4
<i>Halidrys siliquosa</i>	174	<i>Pylaiella litoralis</i>	7
<i>Haplogloia andersonii</i>	4	<i>Sargassum</i> spp.	4, 7
<i>Hesperophycus</i>	4	<i>Scaberia agardhii</i>	7
<i>harveyanus</i>		<i>Scytosiphon lomentaria</i>	7
<i>Heterochondria abietina</i>	4	<i>Spatoglossum</i> spp.	7
<i>Hormosira banksii</i>	7	<i>Turbinaria ornata</i>	4, 7
<i>Hormosira</i> spp.	178	<i>Zonaria crenata</i>	7
<i>Laminaria</i> spp.	4, 7, 172–176		

Table 7.16 Quantitative distribution of major carotenoids in some  
Norwegian Phaeophyceae (mg/kg dry matter) [172]

Alga	Carotene	Violaxanthin	Fucoanthin
<i>Alaria esculenta</i>	210	267	1700
<i>Ascophyllum nodosum</i>	74	140	280
<i>Desmarestia aculeata</i>	190	197	1750
<i>Dictyosiphon foeniculaceus</i>	150	77	411
<i>Fucus serratus</i>	135	180	960
<i>F. vesiculosus</i>	80	162	340
<i>Laminaria digitata</i>	63	110	468
<i>Leathesia difformis</i>	230	200	390
<i>Pelvetia canaliculata</i>	100	294	487
<i>Petalonia fascia</i>	183	164	1060
<i>Pylaiella litoralis</i>	590	2500	5100
<i>Scytosiphon lomentarius</i>	170	182	643

pigment complex has been extracted from *Hormosira* sp. with low concentrations of Triton X-100 [178]. The Phaeophyta which have been examined for carotenoids are given in Table 7.15 and some quantitative data are collected in Table 7.16.

#### (b) *Differential distribution*

In *Fucus vesiculosus*, *F. serratus* and *Ascophyllum nodosum* there is a differential distribution in the male and female forms. The bright orange-yellow colour of the male gametes is due almost entirely to  $\beta$ -carotene (1.13) whilst the olive-green of the ova is due to a mixture of fucoxanthin (1.57) and chlorophyll [174].

### 7.1.9 BLUE-GREEN ALGAE (CYANOBACTERIA)

The blue-green algae should properly be classed with bacteria as prokaryotes which are characterized by the absence of sub-cellular organelles such as mitochondria (and chloroplasts in the case of blue-green algae and photosynthetic bacteria), and by the absence of genetic material located on more than one chromosome in a membrane-bound molecule. However, for convenience, we shall consider them along with the conventional algae.

#### (a) *Distribution*

The characteristic carotenoids common to the blue-green algae are  $\beta$ -carotene (1.13) [179], echinenone (1.22) [180, 181] [= myxoxanthin [182, 183], = aphanin [184, 185]], zeaxanthin (1.18) [179, 181] and myxoxanthophyll (1.20) [179, 181, 183].  $\beta$ -Carotene is normally the only carotene present [179] and blue-green algae apparently cannot make  $\epsilon$ -end groups (see Chapter 1). However, traces, of  $\gamma$ -carotene (1.38) and lycopene (1.11) have recently been found in a hot spring Cyanophyte, a species of *Oscillatoria* [186] and  $\beta$ -zeacarotene (2.28) in a laboratory culture of *Spirulina platensis* although it was not present in cultures collected from Lake Tchad [187]. Mutatochrome (5,8-epoxy- $\beta$ -carotene) is occasionally encountered (e.g. in *Oscillatoria agardii*) [188] as is isocryptoxanthin (1.35) (*Oscillatoria* spp.) [186]. Recent work has indicated that the diketo carotenoid canthaxanthin (1.40) is also quite widely distributed [126, 189]. The previously reported pigment aphanicin [184, 185] has now been identified as canthaxanthin (1.40) [181] but it is not yet clear what relationship the pigments from *Calothrix scopulorum*, described fifty years ago as myxorhodin- $\alpha$ , myxorhodin- $\beta$  and calorhodin [95], have with carotenoids known to occur in other blue-green algae.

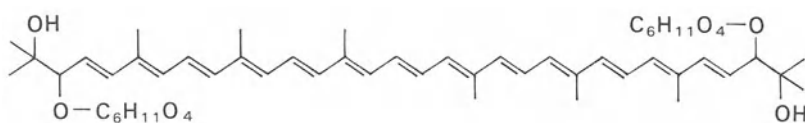
The keto-carotenoid echinenone (1.22) is unique in normal photosynthetic cells of blue-green algae, whilst myxoxanthophyll is a carotenoid glycoside, a

group otherwise found only in some non-photosynthetic and photosynthetic bacteria (Chapters 9 and 10), but not in other algae. As originally isolated myxoxanthophyll was a mixture of glycosides, but the rhamnoside of myxol (the aglycone) is the major component and it is this compound which is now referred to as myxoxanthophyll [182]. The two other components present in the original mixture are myxol-2'-O-methyl-methylpentoside and myxol-2'-glucoside [190]. Myxoxanthophyll is present in all blue-green algae so far examined except *Phormidium* spp. [189, 191] although there is one report [89], later denied [192], that it is also absent from *Anacystis nidulans*. The early report that myxoxanthophyll was absent from the heterocysts of *Anabaena cylindrica* [193, 194] has now been ruled out. The qualitative distribution is the same in vegetative cells and heterocysts of *Anabaena* [195].

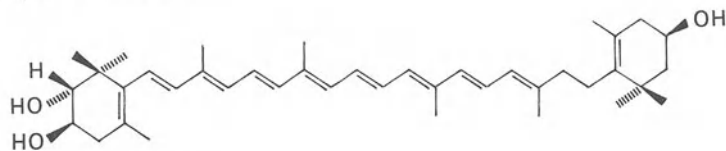
Oscillaxanthin (7.18) first isolated from *Oscillatoria rubescens* [183] is an acyclic diglucoside [181]. Aphanizophyll from *Aphanizomenon flos-aquae* [179] is probably 4-hydroxymyxoxanthophyll [196].

Caloxanthin (7.19) and nostoxanthin (7.20) have been found only in *Nostoc communa*, *Calothrix parietine* and *Synechococcus elongalis* [89, 128] and *A. nidulans* [89, 128, 197, 198]. The structures and absolute configuration of these pigments have recently been clarified [198]. It is important to note that the chirality at C-2 and C-2' is opposite to that in the  $\beta$ -carotene-2,2'-diol found in the green alga *Trentepohlia* (Section 7.1.1). Caloxanthin is probably identical with the trihydroxy- $\beta$ -carotene reported in *Phormidium* spp. [189] and with 'unknown 3' in *Phormidium persicinum* [192]. No epoxy carotenoids were found in a survey of ten blue-green algae [199].

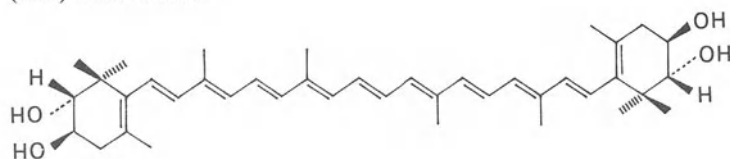
The surface algal mat of a Californian absolute sub-tropical desert, which consists mainly of a mixture of filamentous Nostocinales (*Schizothrix* spp.,



(7.18) Oscillaxanthin



(7.19) Caloxanthin



(7.20) Nostoxanthin

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Table 7.17 Carotenoids in blue-green algae. Major pigments are in *italics*; pigments indicated by numbers in brackets are present only in traces

	<i>Pigments</i>	<i>Ref.</i>
<b>NOSTOCALES</b>		
<b>Oscillatoriaceae</b>		
<i>Athrospira</i> spp. <sup>a</sup>	1, 2, 3, 4, 5, 6, 7	203, 206, 207
<i>Hydrocoleum</i> spp.	1, 3, 4, 6	4
<i>Microcoleus paludosus</i>	1, 4, 6, 8	128
<i>M. vaginatus</i>	1, 4, 6, 8	179
<i>Oscillatoria agardhii</i>	1, 2, 3, 4, 5, 6, 7, 9, 22, 23	188, 199
<i>O. amoena</i>	1, 2, 3, 4, 6, 7	207
<i>O. limosa</i>	1, 2, 3, 4, 10, 11, 12, 13, 19	190
<i>O. princeps</i>	1, 4, 6, 7, 20, 21, 22	208
<i>O. rubescens</i>	1, 2, 3, 4, 5, 6, 7	203, 209
<i>O. tenuis</i>	1, 4, 6, 10, 14, 19	128
<i>Phormidium autumnale</i>	1, 3, 4, 6	4
<i>P. ectocarp</i>	1, 3, (4), (8), 15	91
<i>P. foveolarum</i>	(a) 1, 2, 3, 4, 6, 10, 19 (b) 1, 3, 4, 6, 9, 10, 19	128 189
<i>P. luridum</i>	1, 3, 4, 6, 9, 10, 19	189
<i>P. persicinum</i>	(a) 1, 3, (4), (8), 15, 16, 18 (b) 1, 3, 4, 9, 15	191 189
<i>Spirulina geitleri</i> <sup>a</sup>	2	187
<i>Spirulina maxima</i>	1, 4	212
<i>Spirulina platensis</i> <sup>c</sup> <i>f. granulata</i>	1, 2, 3, 4, 5, 6, 10, 23	210, 211
<i>Spirulina platensis</i> <sup>c,d</sup>	1, 2, 3, 4, 23, 24	187, 211
<b>Nostocaceae</b>		
<i>Anabaena aerulosa</i> <i>oscillatorioidea</i>	1, 3, 4, 6, 10, 11, 19	128
<i>A. cylindrica</i>	(a) 1, (3), 4, 6, 19 (b) 1, 4, 6, 10, 12, 19	179 128
<i>A. flos-aquae</i>	1, (3), 4, 6, 10, 12, 19	189
<i>Anabaena</i> sp.	1, 3, 4, 6	4
<i>A. variabilis</i>	(a) 1, 4, 6, 10 (b) 1, 3, 4, 6, 19	128, 179 191
<i>Aphanizomenon flos-aquae</i>	1, 4, 6, 9, 10, 14, 19	181
<i>Chlorogloeia fritschii</i> <sup>e</sup>	1, 2, 3, 4, 5, 6, (?)7, 9, 10, 16, 17, 20, 23, 24	211A
<i>Cylindrospermum</i> sp.	1, (3), 4, 6	179
<i>Hormothamnion</i> <i>enteromorphoides</i>	1, 3, 4, 6	4
<i>Nostoc commune</i>	1, 3, 4, 6, 8, 10, 16, 17, 19	128, 147, 198
<i>N. muscorum</i>	1, 3, 4, 6	4
<i>Nostoc</i> sp.	1, (3), 3, 6	179

	Pigments	Ref.
Scytonemataceae		
<i>Tolypothrix tenuis</i>	1, 3, 4, 6, 10, 19	128
Rivulariaceae		
<i>Calothrix parietina</i>	1, 3, 4, 6, 8, 10, 16, 17, 19	128
CHROOCOCCALES		
Chroococcaceae		
<i>Anacystis nidulans</i> <sup>f</sup>	(a) 1, 2, 3, 16, 17 <sup>a</sup> (b) 1, 4, 6, 10, 12 <sup>a</sup>	128, 197, 198, 213 186
<i>Chroococcus</i> sp.	1, 3, 4, 6	4
<i>Coccochloris elabens</i>	1, (3), 4, 6	179
<i>Merismopedia punctata</i>	1, 2, 3, 4, 6, 8, 10	128
<i>Microcystis aeruginosa</i>	1, 2, 3, 4, 6, 7, 8, 10, 14, 15, 19	128
<i>Synechococcus elongatus</i>	1, 3, 4, 6, (15), 16, 17, 19	128
STIGONEMATALES		
Mastigocladaceae		
<i>Mastigocladus</i> sp.	1, 3, 4, 6	179

## Key

- |  |        |   |        |
|--|--------|---|--------|
| 1. $\beta$ -Carotene                           | (1.13) | 13. Oscillol-2,2'-di-(O-methyl)         |        |
| 2. $\beta$ -Cryptoxanthin                      | (1.60) | methylpentoside                         |        |
| 3. Zeaxanthin                                  | (1.18) | 14. Aphanizophyll                       | (7.22) |
| 4. Echinenone                                  | 1.22)  | 15. Trihydroxy- $\beta$ -carotene       |        |
| 5. 4-Keto-3'-hydroxy- $\beta$ -carotene        |        | 16. Caloxanthin                         | (7.19) |
| 6. Myxoxanthophyll                             | (1.20) | 17. Nostoxanthin                        | (7.20) |
| 7. Oscillaxanthin                              | (7.18) | 18. 'Unknown 3'                         |        |
| 8. Isozeaxanthin                               | (7.21) | 19. Astaxanthin                         | (1.68) |
| 9. Mutatochrome (5,8-epoxy- $\beta$ -carotene) |        | 20. $\gamma$ -Carotene                  | (1.38) |
| 10. Canthaxanthin (? = euglenanone)            |        | 21. Lycopene                            | (1.11) |
|  | (1.40) | 22. Isocryptoxanthin                    | (1.35) |
| 11. Myxol-2'-O-methyl-methylpentoside          |        | 23. 4-Keto-3-hydroxy- $\beta$ -carotene |        |
| 12. 4-Keto-myxol-2'-methylpentoside            |        | 24. $\beta$ -Zeacarotene                | (2.13) |

## Notes

<sup>a</sup> No  $\beta$ -carotene.

<sup>b</sup> Also 2,3,3'-trihydroxy- $\beta$ -carotene [214].

<sup>c</sup> Surprisingly,  $\alpha$ -carotene,  $\alpha$ -cryptoxanthin, and lutein reported [187, 211].

<sup>d</sup> Zeaxanthin and 4-keto-3-hydroxy- $\beta$ -carotene only present in natural culture from Lake Chad.  $\beta$ -Zeacarotene only present in laboratory cultures of the same strain.

<sup>e</sup> Also an uncharacterized derivative of echinenone, (?) 2-hydroxymyxoxanthophyll, (?) torulene (3,4-dehydro- $\gamma$ -carotene) and plectanixanthin (1',2'-dihydroxy,1',2'-dihydrotorulene).

<sup>f</sup> Possibly a strain of *S. elongatus*.

*Nostoc muscorum*, *Scytonema hoffmanii*) with smaller number of Chroococcales (*Anacystis* spp., *Coccochloris peniocyctis*) and a few Chlorophyta and algae-containing protozoa, contained mainly echinenone (1.22) and canthaxanthin (1.40) but, unexpectedly, no  $\beta$ -carotene [200].

Two symbiotic blue-green algae (cyanomes) which exist together with the colourless algae, *Cyanophora paradoxa* and *Glaucocystis nostochinearum*, synthesize only  $\beta$ -carotene (1.13) and zeaxanthin (1.18); the two very characteristic pigments of the free living forms of blue-green algae, echinenone (1.22) and myxoxanthophyll (1.20), were absent [201].

As indicated previously, carotenoids with  $\epsilon$ -rings probably do not exist in blue-green algae; very early reports of lutein (1.74) in *Oscillatoria rubescens* [202] have not been confirmed in later work [203]. Mutants in which carotenoid synthesis has been impaired have been described briefly in *A. nidulans* and *Agmenellum quadruplicatum* [204–206].

The known qualitative distribution of carotenoids in blue-green algae is given in Table 7.17.

The quantitative distribution of carotenoids in blue-green algae can vary with cultural conditions but generally the predominating pigments are  $\beta$ -carotene and zeaxanthin (1.18), echinenone (1.22) and myxoxanthophyll (1.20). In some cases  $\beta$ -carotene accounts for 80% of the total pigments [215] whilst in other, rather rare cases it appears to be absent [187]. A typical set of quantitative data is given in Table 7.18.

### (b) Localization

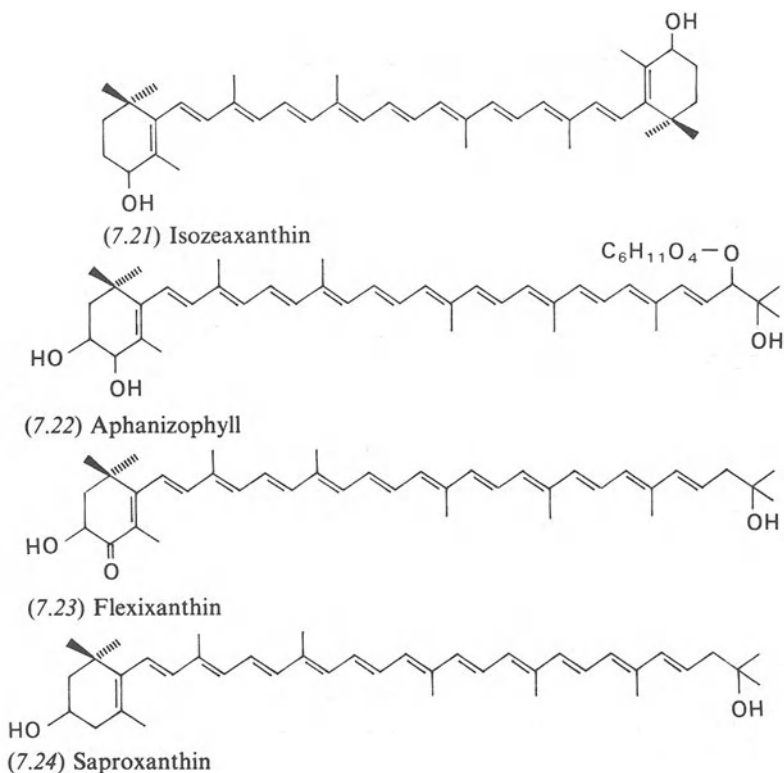
If a homogenate of *Anabaena cylindrica* is separated on a sucrose gradient, the carotenoids appear in the lower fraction [215–217] in which photosystem I activity is concentrated [218] and this is also the situation in *Phormidium luridum* [219]. In *Synechococcus* sp.,  $\beta$ -carotene is concentrated in the 144 000 g fraction of deoxycholate-disrupted organelles; the xanthophylls on the other hand appear in the 10 000 g fraction [220]. In dark-grown *Chlorogloea fritschii* the carotenoids are lipid-soluble and not attached to protein in the reaction centres [211A].

## 7.1.10 PROCHLOROPHYTA

Recently one member of this new order, *Protochloron* sp., a unicellular prokaryotic marine alga associated with didemnid ascidians, has been examined for carotenoids [221B]. The major pigments were  $\beta$ -carotene and zeaxanthin with echinenone,  $\beta$ -cryptoxanthin, mutatochrome and a tri-hydroxy- $\beta$ -carotene as minor components. No pigments with  $\epsilon$ -rings were observed.

Table 7.18 Quantitative distribution of carotenoids in some blue-green algae (figures are percentage of total pigment present)

Alga	$\beta$ -Carotene	Echinenone	Zeaxanthin	Cantha- xanthin	Myxoxan- thophyll	Oscilla- xanthin	Ref.
<i>Oscillatoria rubescens</i> (Oscillatoriaceae)	29	19	8	—	30	10	181
<i>Anabaena flos-aquae</i> (Nostocaceae)	63	13	—	12	4	—	189
<i>Tolypothrix tenuis</i> (Scytonemataceae)	18	40	2	16	23	—	128
<i>Calothrix parietina</i> (Rivulariaceae)	22	23	16	4	13	—	128
<i>Mastigocladus</i> sp. (Mastigocladaceae)	42	39	8	—	11	—	179
<i>Mircocystis aeruginosa</i> (Chroococcaceae)	38	27	11	4	31	1	128
<i>Spirulina platensis</i> (Oscillatoriaceae)	28.5	6.5	16.5	—	37	—	210



#### 7.1.11 COLOURLESS ALGAE

The heterotrophic Chlorophycean phytoflagellate *Polytoma uvella* synthesizes a weakly acidic, incompletely characterized carotenoid, polytomaxanthin; the xanthophyll: carotene ratio in this organism is 4:1 and the total concentration of pigment 0.3–0.4 mg/g dry wt. [221]. In the euglenophyte *Astasia ocellata*,  $\alpha$ -carotene (1.12) is the major carotene and the xanthophylls are almost entirely ketonic and include echinenone (1.22), canthaxanthin (1.40) and probably phoenicopteron (7.4) [222]. *Astasia longa* does not accumulate either phytoene, phytofluene or coloured carotenoids [135]. The apochlorotic dinoflagellate *Cryptocodinium cohnii* is rich in carotenoids [223], producing  $\alpha$ - and  $\beta$ -carotenes as its major pigments [112].

Flexibacteria, sometimes considered as non-photosynthetic blue-green algae, synthesize carotenoids closely related to those of blue-green algae. For example, a *Flexibacter* sp. produces flexixanthin (7.23) and *Saprospira grandis* saproxanthin (7.24) [224] both of which have similarities to myxol (Section 7.1.9). *S. grandis* also accumulates phytoene (1.36), phytofluene (1.37),  $\zeta$ -carotene (1.14) and, possibly, a hydroxy- $\zeta$ -carotene [225]. In many *Flexibacter* (*Cytophaga*) species, e.g. *F. elegans* the major pigment is a non-carotenoid



compound – flexirubin – and no carotenoids are formed. However, in *Cytophaga johnsonae* flexirubin is synthesized along with zeaxanthin and traces of  $\beta$ -carotene,  $\beta$ -zeacarotene and phytofluene; no carotenoid glycosides were produced [226]. *Flexithrix dorotheae* synthesizes zeaxanthin (1.18) with the same chirality as that from higher plants [206, 207].

### 7.1.12 QUANTITATIVE DISTRIBUTION

The pattern of quantitative distribution of carotenoids in algae varies considerably not only from phylum to phylum but also in response to cultural and environmental conditions. However in Table 7.19 some typical values are collected together for comparative purposes.

## 7.2 GENERAL FACTORS CONTROLLING SYNTHESIS

### 7.2.1 GENERAL NUTRITIONAL CONDITIONS

It is clear from the discussion in Section 7.1.1(b) that nutritional imbalance, particularly lack of nitrogen, induces many algae to encyst and to synthesize large amounts of extra-chloroplastidic carotenoids so that the cultures turn orange or red [227–232].

Under some conditions, e.g. *Dictyococcus cinnabarinus* growing in submerged culture in the presence of glucose, synthesis of these supernumerary carotenoids can be induced in growing cells [56, 233, 235] but in the case of *Chlorella* nitrogen deficiency merely results in general reduction of carotenoid levels [236]. On the other hand maximum synthesis by the red algae *Pterocladia capillacea* grown in sea water was obtained with  $10^{-4}\text{M}$   $\text{NH}_4\text{NO}_3$  whilst  $10^{-3}\text{M}$  lowers carotenoid levels [234]. Maximum levels in *Poteriochromonas stipitata* are achieved in the presence of  $1 \times 10^{-2}\text{M}$  citrate; the same effect is obtained by addition of  $5 \times 10^{-2}\text{M}$  pyruvate,  $1.2 \times 10^{-2}\text{M}$   $\alpha$ -ketoglutarate or  $1.5 \times 10^{-2}\text{M}$  succinate, fumarate or malate [237]. This stimulation is generally accepted as an additional *de novo* synthesis [231, 233] and not as the result of degeneration of chloroplasts and the subsequent re-utilization of breakdown products particularly of the chlorophylls [234]. Under heterotrophic conditions carotenoid synthesis by *Dictyococcus cinnabarinus* at  $5^\circ\text{C}$  is best with galactose as carbohydrate source; at  $15^\circ\text{C}$ , however, glucose is the most effective carbohydrate [238]. In streptomycin-bleached cells of *Euglena gracilis* the optimum pH for carotenogenesis is 4.0 in the light and 6.0 in the dark [239]. Pigment production in *Chlorella terricola* is stimulated by adding potato juice to the culture medium [240].

The antibiotic Rifampicin had no effect on carotenogenesis in *Chlamydomonas reinhardtii*; thus the DNA of chloroplasts is not required for synthesis of enzymes involved in carotenoid production. However both cytoplasmic and chloroplastic ribosomes are required because both cycloheximide and

Table 7.19 Typical quantitative distribution of major carotenoids in representative algae from different Phyla (mg/g dry wt.)

	CHLORO- PHYTA	CHRYSOPHYTA			PHAEO- PHYTA	RHODOPHYTA		PYRO- PHYTA	CYANO- PHYTA	CRYPTO- PHYTA	EUGLENO- PHYTA	
		<i>Xantho- phyceae</i> <i>Vischeria</i> <i>sp.</i> [150]	<i>Bacillario- phyceae</i> <i>Phaeodactylum</i> <i>bicocutum</i> [122]	<i>Chryso- phyceae</i> <i>Ochromonas</i> <i>stipitata</i> [2]	<i>Laminaria</i> <i>sp.</i> [2]	<i>Bangiodend. Flor- ideae</i> <i>Rhodosorus</i> <i>marinus</i> [87]		<i>Nemalion</i> <i>multifidum</i> [87]	<i>Gymnodinium</i> <i>sp.</i> [122]	<i>Anabaena</i> <i>variabilis</i> [179]	<i>Cryptomonas</i> <i>sp.</i> [2]	<i>Euglena</i> <i>gracilis</i> [2]
$\alpha$ -Carotene (1.12)	0.09	—	?	—	—	—	0.02	—	—	—	0.18	—
$\beta$ -Carotene (1.13)	0.33	3.27 <sup>a</sup>	0.31 <sup>b</sup>	0.55	0.73	0.09	0.10	0.10	1.90	—	—	0.74
Echinonone (1.22)	—	—	—	—	—	—	—	—	—	—	—	0.31
Lutein (1.74)	1.11	—	—	—	—	0.03	0.40	—	1.56	—	—	—
Zeaxanthin (1.18)	—	—	—	0.78	0.14	0.29	—	—	0.22	—	—	—
Violaxanthin (1.58)	0.22	—	—	1.14	0.21	(?)	0.05	—	—	—	—	0.28
Neoxanthin (1.47)	0.21	—	—	—	—	—	—	—	—	—	—	—
Fucoxanthin (1.57)	—	—	5.35	2.05	2.98	—	—	—	—	—	—	—
Diadinoxanthin (2.28)	—	1.74	0.22	—	—	—	—	0.19 <sup>d</sup>	—	—	—	4.24
Alloxanthin (1.46)	—	—	—	—	—	—	—	—	—	—	2.22	—
Peridinin (1.71)	—	—	—	—	—	—	—	—	0.52	—	—	—
Myxoxantho- phyll (1.20)	—	—	—	—	—	—	—	—	—	1.90	—	—
Unknown or minor	0.25	trace	—	0.23	0.04 <sup>e</sup>	—	0.05	—	1.90	0.24 <sup>e</sup>	1.41 <sup>f</sup>	—
TOTAL	2.21	5.01	5.88	4.75	4.10	0.41	0.62	0.81	5.58	2.64	6.98	—

Notes

<sup>a</sup> In other Heterokonts  $\beta$ -carotene levels are lower.

<sup>b</sup> Total carotenes.

<sup>c</sup>  $\epsilon$ -Carotene (7.2) + antheraxanthin (1.24).

<sup>d</sup> Dinooxanthin (7.8) also present.

<sup>e</sup> Crocoxanthin (7.11).

<sup>f</sup>  $\beta$ -Cryptoxanthin (1.60)  $\beta$ -cryptoxanthin 5,6-epoxide, astaxanthin ester (1.68).

spectinomycin inhibited carotenogenesis. It would seem that  $\beta$ -carotene synthesis is completely dependent on functional cytoplasmic ribosomes [241]. Carotenoid levels in *Scenedesmus obtusiusculus* increase during the growth phase but decline during the sporulating phase [242]. Levels during the cycle of synchronous cultures of *Chlamydomonas reinhardtii* have been reported [243] and the maximum content appears after 20–23 days in mass cultures of *Dunaliella salina* [244].

### 7.2.2 MINERAL NUTRITION

The general claim that lack of  $\text{Mg}^{2+}$ ,  $\text{SO}_4^{2-}$  or  $\text{PO}_4^{3-}$  causes an accumulation of carotenoids in algae [245] was not confirmed in *Trentepohlia aurea* [70]; deficiency of  $\text{K}^+$  and  $\text{Ca}^{2+}$  also has no effect on *T. aurea*. In *Ankistrodesmus*, however, lack of  $\text{PO}_4^{3-}$ ,  $\text{SO}_4^{2-}$ ,  $\text{Ca}^{2+}$  and  $\text{Fe}^{3+}$  does stimulate carotenogenesis, although  $\text{Mg}^{2+}$  and  $\text{Mn}^{2+}$  deficiency was innocuous [246]. When *Scenedesmus obtusiusculus* was grown on a  $\text{Ca}^{2+}$ -deficient medium and then transferred to a culture containing calcium, the carotenoid content of the cells was markedly increased within 15–33 h [247]. Copper deficiency increases carotenoid production in *Oscillatoria rubescens* whilst lack of iron, cobalt, boron, manganese or zinc lowers the levels [248]. A low phosphate medium stimulated carotenogenesis threefold in the *Euglena* mutant SML1 [249]. In NaCl concentrations of about 5%, *Dunaliella salina* synthesizes a considerably increased amount of  $\beta$ -carotene [250–253], whilst the other normal chloroplast pigments do not alter [251]; this is obviously an effect similar to that noted in Section 7.1.1(b) when certain green algae are starved of nitrogen. Fluoride ( $500 \text{ mg dm}^{-3}$ ) reduces carotenogenesis in the green algae as does  $\text{Hg}^{2+}$  and  $\text{Zn}^{2+}$  in *Callithamnion corymbosum* and *Enteromorpha intestinalis*; the effect of the metals can be overcome by EDTA, Triton B or  $\text{FeSO}_4$  [255]. Fluoride ( $500 \text{ mg dm}^{-3}$ ) reduces carotenogenesis in the green algae *Chlorella vulgaris*, *Scenedesmus quadricauda* and *Dyctosphaerium pulchellum* [254]. The effect of silicon deficiency on carotenoid production by the diatom *Navicula pelliculosa* has been described [256].

### 7.2.3 LIGHT

#### (a) Intensity

When *Euglena* spp. are cultured heterotrophically in the dark carotenoid synthesis is much reduced [257–259]; if dark-grown cells are sub-cultured in the dark the cultures eventually become almost colourless because the cells cease to form chloroplasts. No neoxanthin is present in dark-grown *Euglena* but it appears along with chlorophyll on illumination when chloroplasts develop [260]; blue light appears to be most effective [239]. On the other hand, some algae, e.g. *Chlorella*, *Chlamydomonas*, will produce functional chloroplasts, and thus carotenoids, in the dark. The amount in young dark-

grown cultures of *Chlorella* is less than in corresponding light cultures but in old cells the situation is reversed [261] and prolonged darkness (2 months) had little effect on the carotenoid level [262]. A dark-grown mutant (y-1) of *Chlamydomonas reinhardtii* cannot synthesize chlorophylls in the dark, but synthesizes the normal range of carotenoids, although  $\beta$ -carotene (1.13) is quantitatively greater than in the native cells. On illumination and formation of chloroplasts, lutein (1.74) and neoxanthin (1.47) increase to a much greater extent than do  $\beta$ -carotene and violaxanthin (1.58) [38]. Dark-grown cells of the pennate diatom *Cylindrotheca fusiformis* produced all the carotenoids observed in light-grown cells except diadinoxanthin (2.28) [263] and, in the case of dark-grown *Scenedesmus obliquus* neoxanthin (1.47) was missing [264] as in dark-grown *Euglena*.

Under naturally occurring autotrophic conditions, changes in light intensity appear to have little effect on carotenoid production, because samples of the same algae taken at various depths in a mid-west lake differed only slightly in their carotenoid content [265]. Similarly experiments with the blue-green alga *Oscillatoria rubescens* revealed no effect on carotenogenesis of variation of light intensities between 800 and 5420 lx [266]. Increasing the light intensity falling on cultures of *Anacystis nidulans* from 1 to 50 W m<sup>-2</sup> increased the carotenoid levels relative to those of chlorophyll [268]. However, *in vitro* cultures of the red alga *Petroglossum nicaense* respond to high light intensity by synthesizing more carotenoids [267]. Under autotrophic conditions high light intensity increases carotenogenesis in *Chlorella* in the presence of optimal amounts of nitrogen; in nitrogen deficiency the levels are lower; when the cells are growing heterotrophically in the presence of glucose no light effect is observed [268]. Reports of qualitative differences in pigment production under the influence of different light regimes have been published; under high light intensity *Chlorella pyrenoidosa* produces more  $\beta$ -carotene (1.13) than  $\alpha$ -carotene (1.12), whilst the reverse situation exists in cultures grown under low intensities [269]. *Nitzschia closterium* produces less diadinoxanthin (2.28) when cultured under 'snow-white' fluorescent lamps than under neon tubes although the other pigments are unaffected [123]. *Chlorogloea fritschii* produces in darkness more echinenone and much more myxoxanthophyll than in the light [211A]. It is claimed that pulsed light increases carotenoid production in *Chlorella* [270].

### (b) *Quality*

There are a few reports on the effect of quality of light; green light stimulates carotenogenesis in *Cyclotella nana* and *Dunaliella tertiolecta* [271]. In *Chlorella pyrenoidosa* the total carotenoid levels are the same under all light regimes but the carotene:xanthophyll ratio is higher in red and green light than in blue and white. Neoxanthin (1.47) levels are highest in white light and lowest in red light [272]. Diatoms, dinoflagellates and brown algae do not show marked

changes in carotenoids in response to varying light intensities when measured in relation to chlorophyll production [273] but important variations in the ratio of photosynthetically active to inactive carotenoids are observed [274]. This effect is also observed in green algae [275, 276].

(c) *Effect on mutants*

A number of carotenoid deficient mutants of *Chlorella* are killed by a combination of light and oxygen (Chapter 3). Mutant 5/520 synthesizes a series of acyclic colourless polyenes in the dark: on anaerobic illumination cyclic carotenes are formed and red light is the most effective stimulator [277]. The effect of light is still unexplained because as noted previously, light is not required for carotenogenesis in native *Chlorella*. Hydroxylation of carotenoids in the mutant strain is light-independent because if the cells illuminated anaerobically are returned to darkness and then exposed to oxygen then xanthophylls are formed [278, 279]. The small amount of prolycopene [49] and proneurosporene present in dark-grown cells are isomerized by light, which they themselves absorb, into the all-*trans* isomers in the presence of oxygen. In the absence of oxygen light absorbed by the small amounts of chlorophyll formed in the dark will also mediate this photo-isomerization [280]. In a chlorophyll-deficient mutant of *Chlorella vulgaris* constant irradiation with blue light stimulates carotenoid synthesis, the half-saturation effect being observed at 1000 erg/cm<sup>2</sup>/sec. Maxima were observed at 370 and 465 nm in the action spectrum which resembled that for a number of other responses such as oxygen uptake and carbohydrate consumption [281]. In *Euglena* mutants light stimulates synthesis of coloured carotenes apparently at the expense of colourless precursors [135]. A mutant of *Scenedesmus obliquus* has been obtained which synthesizes mainly  $\zeta$ -carotene in the dark. On illumination, it rapidly develops functional chloroplasts which contain the usual array of carotenoids which have been synthesized from the preformed  $\zeta$ -carotene [84–86, 281A].

(d) *Extra-plastidic synthesis*

Light is not essential for the formation of astaxanthin (1.68) in encysting *Haematococcus pluvialis* [62] but it is stimulatory [60] as it is in *Chlorococcum wimmeri* [282]. Extra-plastidic synthesis of carotenoids is independent of light intensity in *Trentepohlia aurea* [70] and other *Chlorophyceae* [47].

#### 7.2.4 TEMPERATURE AND PH

When the temperature of cultivation is increased from 17°C to 38°C the carotenoid content of *Anacystis nidulans* is increased; all components except echinenone contribute to this increase [283]. Similarly high temperatures

stimulate carotenogenesis in *Chlorella* grown under autotrophic conditions [268]. A pH of 7.0 is best for carotenogenesis in *C. vulgaris* [284].

### 7.2.5 INHIBITORS AND ANTIBIOTICS

The effects of some well-known metabolic inhibitors and antibiotics on carotenogenesis in various algae are summarized in Table 7.20.

### 7.2.6 SEASONAL FACTORS

The pattern of seasonal variation in carotenoid content varies considerably with different algae. For example, in Norwegian waters the levels in *Fucus serratus* are reasonably constant whilst those for *Pelvetia canaliculata* undergo considerable fluctuation (Fig. 7.1) [172]. In the latter the minimum value coincides with the period of maximum fructification [294]. In a *Peridinium* bloom in an Israeli lake there were considerable changes in the carotenoids although the species of algae present remained constant [295].

Table 7.20 Effect of inhibitors and antibiotics on carotenoids of algae

Agent	Alga	Effect	Ref.
Antimycin A	<i>Anacystis nidulans</i>	Little	285
Cycloheximide	<i>Chlamydomonas reinhardi</i>	Inhibits	241
Dimethyldioxane	<i>Dunaliella salina</i>	Stimulates	286
4,6-Dinitro- <i>o</i> -cresol	<i>Poteriochromonas stipitata</i>	Inhibits	287
2,4-Dinitrophenol	<i>Dunaliella minuta</i>	No effect	288
	<i>D. salina</i>	Stimulates	289
Diphenylamine	<i>Anabaena variabilis</i>	Inhibits but myxoxanthophyll increases	290
Diuron	<i>A. nidulans</i>	Inhibits	285, 289, 291
Hydroxylamine	<i>A. nidulans</i>	Inhibits	289
Rifampicin	<i>C. reinhardi</i>	No effect	241
SAN 9789	<i>Euglena</i>	Inhibits with accumulation of phytoene	{ 140 292
	<i>Ankistrodesmus braunii</i>		
SAN 6709	<i>Ankistrodesmus braunii</i>	Inhibits	293
Basf 44521	<i>Ankistrodesmus braunii</i>	Inhibits	293
Spectinomycin	<i>C. reinhardi</i>	Inhibits	285

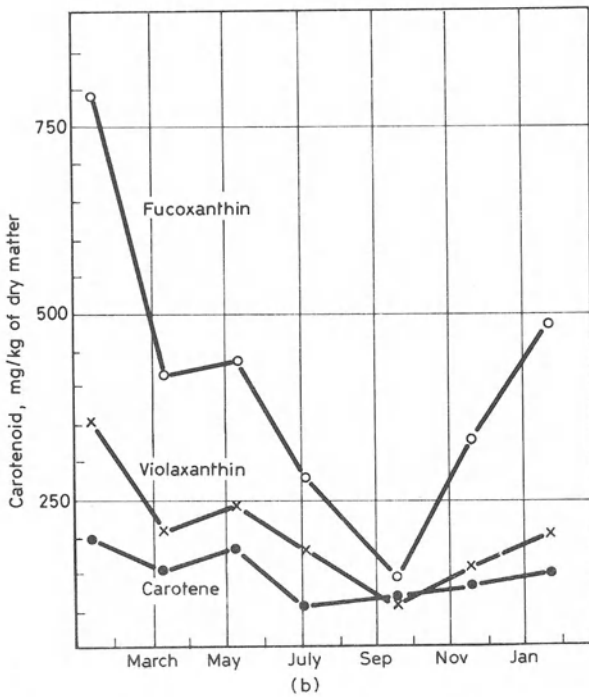
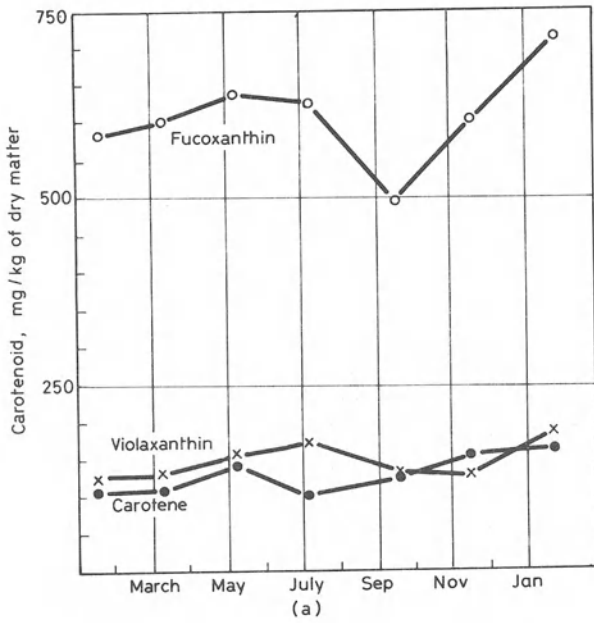


Fig. 7.1. Seasonal variations in carotenoid content of *Fucus serratus* (a) and *Pelvetia canaliculata* (b) growing in Norwegian waters [172].

## 7.2.7 MISCELLANEOUS

When a colourless strain of *Dunaliella salina* is grown together with a normal strain the carotenoid level in the latter is reduced [296].

## 7.3 CAROTENOIDS, ALGAL TAXONOMY AND EVOLUTION

There is little doubt that the basic biosynthetic mechanism involved in assembling the C<sub>40</sub> skeleton (phytoene, (1.36)) of carotenoids is the same in all organisms. It is also probable that the desaturation of phytoene to lycopene (1.11) is the major pathway leading to cyclic carotenoids. It is the further metabolism of lycopene, varying as it does from phylum to phylum, which can be used to help algal taxonomy and theories on algal evolution. These further changes, in algae, can be related in the main to the following enzymes: (a)  $\beta$ -cyclase – which forms the  $\beta$ -ring of cyclic carotenoids: (b)  $\epsilon$ -cyclase – which forms the  $\epsilon$ -ring: (c) hydroxylases inserting OH at C-3 and C-3': (d) 5,6-epoxidase: (e) 5,6-epoxide isomerase, forming allenes such as neoxanthin (1.47). Also involved are reactions hydroxylating in-chain methyl groups to form pigments such as in loroxanthin (7.1), forming acetylene linkage as in diadinoxanthin (2.28), and inserting in-chain keto groups as in siphonaxanthin (2.17).

With this basic information in mind an evolutionary scheme for algae can be drawn up (Fig. 7.2). If one assumes that the primitive precursor can synthesize acyclic carotenoids then the first phylum to emerge would be the blue-green algae. They possess only  $\beta$ -cyclase (a) and produce  $\beta$ -carotene (2 $\beta$ -rings) and its derivatives and derivatives of  $\gamma$ -carotene (one  $\beta$ -ring and one acyclic end); however one of the main  $\beta$ -carotene derivatives is echinenone in which an oxygen has been inserted at C-4; this is a specialized reaction which rarely if ever occurs in the chloroplasts of other algae.

The other major pigment is the monocyclic carotenoid glycoside myxoxanthophyll. This type of carotenoid does not occur in other algae but in containing the C-1,2 double bond hydrated and the resulting hydroxyl group glycosylated it is structurally very similar to many carotenoids of the photosynthetic bacteria; this correlation would support the view held that blue-green algae are prokaryotes and should no longer be classified within the algae.

The red algae could have evolved from blue-greens by developing enzyme 2, an  $\epsilon$ -cyclase (to produce  $\alpha$ -carotene derivatives) in some but not all cases. At the same time they have lost or repressed the ability to insert oxygen at C-4 and to form the enzyme concerned with glycosylating acyclic carotenoids. Alternatively the blue-green algae may have evolved the latter enzymes after the red algae had branched off.

The main pigment development in the evolution of the Chlorophyta from



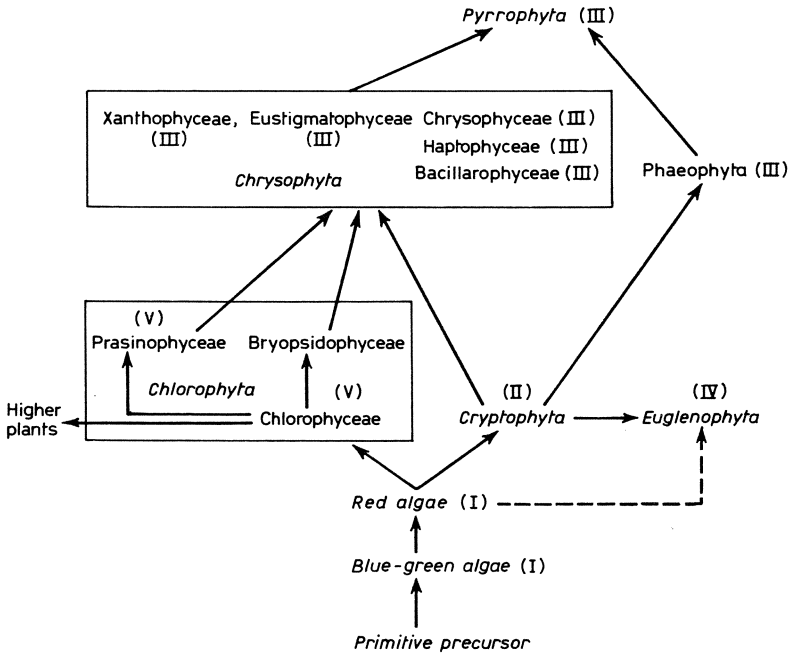


Fig. 7.2. A proposal for algal evolution based on carotenoid distribution (Roman numerals in brackets indicate thylakoid type [297].)

the Rhodophyta is the development of two new enzymes the 5,6-epoxidases and the 5,6-epoxide isomerases. These would account for the constant appearance of antheraxanthin (1.24), violaxanthin (1.58) and neoxanthin (1.47) in this phylum. (There have been only rare reports of 5,6-epoxides in red algae.) However further development within the phylum has occurred in the Classes Bryopsidophyceae and the Prasinophyceae. Specimens of the orders Derbsiales, Codiales, Caulerpales and Dichotomosiphonales so far examined synthesize a pigment more oxygenated than the normal chlorophyte pigments; this is siphonaxanthin (2.17) in which the C-19 in-chain methyl group is oxidized to a hydroxymethyl group and a conjugated ketone appears at C-8. The oxidized in-chain methyl group also appears in loroxanthin (no keto group at C-8) which occurs spasmodically in the Chlorophyceae as well as in the Bryopsidophyceae (see Table 7.2). The C-8 keto group is also found in fucoxanthin, the characteristic pigment of Phaeophyta, and some Chrysophyta. A possible mechanism for the formation of the keto group at C-8 is given in Fig. 7.3. As yet it has no experimental support and a thorough search in one organism, *Codium fragile*, failed to reveal the presence of an acetylenic carotenoid [25] required as an intermediate in the proposal shown in Fig. 7.3. Also very frequently associated with siphonaxanthin is siphonein which is siphonaxanthin esterified probably at C-3, with long chain fatty

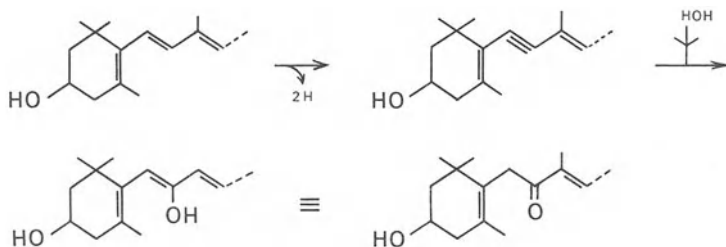


Fig. 7.3. Possible mechanism for forming in-chain ketones from acetylenic carotenoids.

acids. The existence of esterified xanthophylls in chloroplasts is rare, but fucoxanthin is acetylated at C-3 which gives added support to the possibility that the Bryopsidophyceae are rather closely related to the Phaeophyta. Of the other orders of Bryopsidophyceae siphonaxanthin and/or siphonein are sporadically found in the Cladophorales and Siphonocladales, but have not yet been observed in the Sphaerophyceae. They are found frequently in the Class Prasinophyceae. One Chlorophyceae, *Microthamnion knetzingianum* has been reported to make siphonaxanthin.

The green plants which have evolved from Chlorophyta must have had ancestors in Classes other than those just considered, probably the Chlorophyceae, because no higher plants produce pigments similar to loroxanthin and siphonaxanthin.

The next important evolutionary step would seem to be the appearance of the acetylenic bond in carotenoids and these pigments, which would appear to be formed as the result of direct desaturation of pigments such as lutein (1.74) and zeaxanthin (1.18) with no additional structural variations, are characteristic of the Cryptophyta, which can be considered as evolving directly from the red algae. The Euglenophyta could, in turn, have developed from the Cryptophyta basically in a manner analogous to that by which the Chlorophyta developed from the Rhodophyta. The possibility also exists that the Euglenophyta might have evolved from the blue-green algae without the intermediation of the red algae because they cannot synthesize  $\epsilon$ -rings and this is also characteristic of the Cryptophyta. On the other hand they might have evolved from specific red algae which themselves never evolved the ability to synthesize  $\epsilon$ -rings. The various schemes which suggest that they have evolved from Chlorophyta have not explained the fact that they cannot synthesize  $\epsilon$ -rings in their carotenoid pigments.

When the phylum Chrysophyta is considered there is only one clear picture relating the five classes. All except the Chrysophyceae synthesize acetylenic carotenoids and a re-consideration of the early work on this order could well indicate that the pigments present were wrongly identified as antheraxanthin and fucoxanthin. The appearance of acetylenic carotenoids suggests that the Chrysophyta arose from the Cryptophyta rather than from the Chlorophyta.

On the other hand evolutionary developments have produced heteroxanthin (7.15) in the Xanthophyceae, which involves in-chain methyl oxidation characteristic of loroxanthin (7.1), and fucoxanthin (1.57) in the Chrysophyceae, Bacillariophyceae and Haptophyceae, which involves formation of a conjugated keto group characteristic of siphonaxanthin (2.17). Vaucherianxanthin (7.16), which accompanies heteroxanthin in the Xanthophyceae and Eustigmatophyceae, could have been formed by a loroxanthin-type oxidation of neoxanthin (1.47) rather than from lutein (1.73). All these observations indicate a development from the Chlorophyta via the Briopsidophyceae. No  $\epsilon$ -rings are observed in any of the Chrysophyta, which suggests that they are not formed from the Chlorophyta. The Phaeophyta and the Pyrrophyta in which the major pigments are fucoxanthin (1.57) and peridinin (1.71), respectively, also do not make  $\epsilon$ -rings. If peridinin, in spite of the loss of three in-chain carbon atoms, is an oxidation product of fucoxanthin, then the Pyrrophyta might be considered as evolving from fucoxanthin-producing ancestors i.e. Chrysophyta or Phaeophyta. The Chloromonadophyta, as far as they have been examined, appear to have very similar pigments to those in the Xanthophyceae.

There is clearly a considerable degree of inter-phylum reaction which has developed in these more advanced algae and much more work is necessary before the problem of pigment distribution is finally settled. All the views just expressed, seen through the orange-coloured spectacles of one carotenoid biochemist (others have slightly different views [296]) must only be considered a minor contribution to the problem and should be balanced by consideration of other relevant parameters which have been discussed in detail by Klein and Cronquist [297], but it is interesting to note that the correlation of chloroplast ultrastructure, as exemplified by thylakoid-type, and carotenoid pigment is impressive [298].

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Table 8.1 Carotenoid distribution in Phycomycetes  
(the figures in italics indicate the major components)

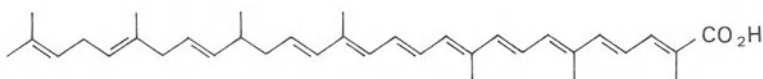
<i>Fungus</i>	<i>Pigments</i>	<i>Ref.</i>
<b>Chytridiales</b>		
<i>Chytridium</i> spp.	1	1-3
<i>Chytriomyces aureus</i>	2	4
<i>C. hyalinus</i>	2	4
<i>Cladochytrium replicatum</i>	2, 3	5
<i>Pleotrachelus fulgens</i>	1	2, 3
<i>Rhizophlyctis rosea</i>	3, 4	6
<b>Blastocladales</b>		
<i>Allomyces arbuscula</i>	3	7
<i>A. javanicus</i>	2, 4, 5, 6, 7	7, 8
<i>A. macrogynus</i>	3	7, 9
<i>A. moniliformis</i>	3	7
<i>Blastocladiella</i> spp.	3	10
<i>Blastocladiella variabilis</i>	10	11
<b>Mucorales</b>		
<i>Blakeslea trispora</i>	2, 3, 5, 6, 7, 8	12-14
<i>Choanephora circinans</i>	2	15-17
<i>C. cucurbitarum</i>	2	15-17
<i>Mortierella rammiana</i>	2	18
<i>Mucor azygospora</i>	3	19
<i>M. flavus</i>	2	3, 20
<i>M. hiemalis</i>	2	21
<i>M. inaequisporus</i>	2	19
<i>Phycomyces blakesleeanus</i> <sup>a</sup>	2, 3, 4, 5, 6, 7, 8, 9	22-29
<i>P. nitens</i>	2, 5, 6, 7	31
<i>Pilaria anomala</i>	2	32
<i>Pilobolus crystallinus</i>	1	2, 3
<i>P. kleinii</i>	2	31
<i>P. oedipus</i>	1	2, 3
<i>Syzygites megalocarpus</i>	2, 3	32

**Key**

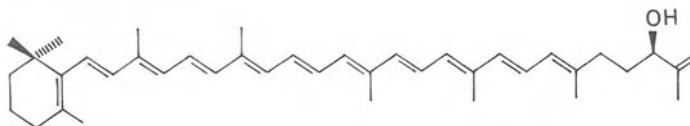
- |                             |                         |        |
|-----------------------------|-------------------------|--------|
| 1. Present but unidentified | 6. Phytofluene          | (1.37) |
| 2. $\beta$ -Carotene        | 7. $\zeta$ -Carotene    | (1.14) |
| 3. $\gamma$ -Carotene       | 8. Neurosporene         | (2.14) |
| 4. Lycopene                 | 9. $\beta$ -Zeacarotene | (2.13) |
| 5. Phytoene                 | 10. 'Carotene'          |        |

**Note**

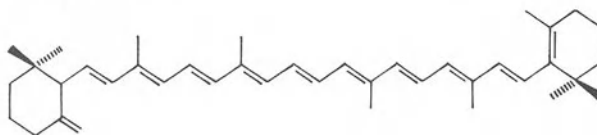
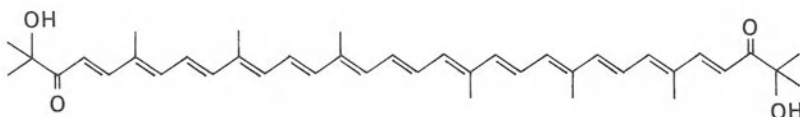
<sup>a</sup> 7',8',11',12'-tetrahydro- $\gamma$ -carotene occurs in cultures grown in presence of diphenylamine [30].



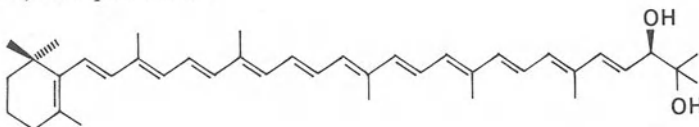
(8.1) Neurosporaxanthin



(8.2) Aleuriaxanthin

(8.3)  $\beta,\gamma$ -Carotene

(8.4) Phillipsiaxanthin



(8.5) Plectanixanthin

photosynthetic bacteria. A hydroxyl group at C-2' at the acyclic end of plectanixanthin is a structural feature of carotenoids in some non-photosynthetic bacteria and is also occasionally found in algae. The taxonomic significance of these new pigments in the Pezizales has still to be fully assessed [54]. The known distribution of carotenoids in Ascomycetes is given in Table 8.2.

### 8.1.3 HETEROBASIDIOMYCETES

The main characteristics of the carotenoid distribution in the Heterobasidiomycetes (Table 8.3) are again the accumulation of hydrocarbons rather than xanthophylls and the appearance of torulene (2.24), once thought to be specific to red yeasts. The xanthophylls, where they occur, are carotenoids with hydroxyl groups at C-3 such as  $\beta$ -cryptoxanthin (1.60), rubixanthin (4.9) and zeaxanthin (1.18). These pigments are characteristic of higher plants and have not been reported in other fungi; perhaps their identification should be checked by modern methods.

Table 8.2 Carotenoid distribution in Ascomycetes  
(figures in italics indicate the major pigment)

<i>Fungus</i>	<i>Pigments</i>	<i>Ref.</i>
<b>Endomycetales</b>		
<i>Saccharomyces</i> spp.	1	34
<b>Plectascales</b>		
<i>Aspergillus giganteus</i>	2	35, 36
<i>A. niger</i>	1	37
<i>Penicillium lapidosum</i>	3	37
<i>P. multicolor</i>	2, 3, 4, 5	39, 40
<i>P. sclerotiorum</i>	2, 3	38
<i>Rhizoctonia zeae</i>	2, 3, 6	38
<b>Protomycetales</b>		
<i>Protomyas inudatus</i>	2, 3	41
<i>P. inouyei</i>	2, 3, 5	40, 41
<i>P. pachydermus</i>	2, 3, 5	40, 41
<b>Sphaeriales</b>		
<i>Leptosphaeria michotii</i>	2, 21	42
<i>Nectria cinnabarina</i> <sup>b</sup>	1, 20	2, 18, 43
<i>Neurospora crassa</i> <sup>a</sup>	2, 3, 5, 6, 7, 8, 9, 10, 11	44–48
<i>N. sitophila</i>	2, 3, 5, 6, 7, 8, 9, 10, 11	44, 46, 49, 50
<i>Polystigma fulvum</i>	1	2
<i>Polystigma rubrum</i>	12(?)	51
<i>Sphaerostilbe coccaphili</i>	1	18
<b>Clavicipitales</b>		
<i>Cordyceps militaris</i>	1	52
<i>Epichloe typhina</i>	2, (3?)	53
<b>Heliotales</b>		
<i>Calycella citrina</i>	2	34, 54
<i>Dasyscyphus bicolor</i>	2, 3	53
<i>Heliotium citrinum</i>	2	54
<i>H. subcorticale</i>	2	54
<i>H. spp.</i>	2, 3	54
<i>Leotia lubrica</i>	2, 3, (9?)	54
<i>Microglossum olivaceum</i>	2, 3	54
<i>Mitrula paludosa</i>	2, 3	54
<i>M. rhemii</i>	2	54
<i>Spathularia flavida</i>	2	23, 54
<i>Trichoscyphella calycina</i>	2	54
<i>T. gallica</i>	2	
<b>Pezizales</b>		
<i>Aleuria aurantia</i> ( <i>Peziza aurantia</i> )	2, 3, 9, 13, 14	46, 54–56
<i>A. rhenana</i>	2, 3, 9	54
<i>Anthracobia melaloma</i>	2, 3, 8, (9?)	54, 57
<i>Ascobolus</i> spp.	1	2
<i>Caloscypha fulgens</i>	2, 3, (4?), 5, 8, 9, 10, 1	54, 58, 59, 60

<i>Fungus</i>	<i>Pigments</i>	<i>Ref.</i>
<i>Cheilymenia crucipila</i>	3, (9?)	54
<i>C. theleboloides</i>	3	54
<i>Cookeina sulapes</i> <sup>b</sup>	(4?), 5, 11, 16, 19	54, 59
<i>Coprobia (Humaria) spp.</i>	1	57, 61
<i>Geopyxis carbonaria</i>	9	54
<i>G. maialis</i>	2, 3, 9	54
<i>Leucoscypha rutilans</i>	2, 3, 17	54
<i>Melastiza chateri</i>	2, 3, 13, 14	54
<i>M. greletti</i>	2, 3, 13, 14	54
<i>Octospora calichora</i>	14	54
<i>O. leucoloma</i>	2, 3, 17	54
<i>O. rubricosa</i>	2, 3, 17	54
<i>Peziza aeruginosa</i>	1	2, 3
<i>P. bicolor</i>	1	2, 3
<i>Pithya vulgaris</i>	2, 18	54
<i>Phillipsia carminea</i>	2, 5, 14, 16, 18	54, 58
<i>P. carnicolor</i>	16	54
<i>Plectania coccinea</i>	2, 3, 14, 17	54, 62, 63
<i>Pyronema confluens</i>	2, 3, 9, 19	64
<i>P. omphalodes</i>	1	54
<i>Pulvinula constellatio</i>	2, 3, 17	54
<i>Scutellina ampullacea</i>	3, 9	54
<i>S. arenosa</i>	3, 9	54
<i>S. setosa</i>	2, 3	54
<i>S. scutellata</i>	3, 9	54
<i>S. superba</i>	3, 9	54
<i>S. trechispora</i>	3, 9	54
<i>S. umbrarum</i>	3, 8, 9	54
<i>Sowerbyella radricula</i>	2, 3	54
<i>S. unicolor</i>	2, 3, 9	54

## Key

- |                               |   |        |
|-------------------------------|---|--------|
| 1. Present but not identified | 11. Neurosporaxanthin                       | (8.1)  |
| 2. $\beta$ -Carotene          | (1.13) 12. Lycoxanthin (?)                  | (5.11) |
| 3. $\gamma$ -Carotene         | (1.38) 13. Aleuriaxanthin                   | (8.2)  |
| 4. $\beta$ -Zeaxarotene       | (2.13) 14. 2,3-Didehydroplectanixanthin     |        |
| 5. Lycopene                   | (1.11) 15. $\beta,\gamma$ -Carotene         | (8.3)  |
| 6. Phytofluene                | (1.37) 16. Phillipsiaxanthin                | (8.4)  |
| 7. $\zeta$ -Carotene          | (1.14) 17. Plectanixanthin                  | (8.5)  |
| 8. Neurosporene               | (2.14) 18. 2',3'-Didehydrophillipsiaxanthin |        |
| 9. Torulene                   | (2.24) 19. Torularhodin                     | (1.21) |
| 10. 3,4-Didehydrolycopene     | 20. <i>cis</i> -Phytoene                    |        |

## Notes

<sup>a</sup> A pigment originally designated  $\theta$ -carotene is a mixture of  $\zeta$ -carotene and its isomer 7,8,11,12-tetrahydrolycopene.

<sup>b</sup> Reported as the methyl ester which is almost certainly an artefact [65].

Table 8.3 Carotenoid distribution in the Heterobasidiomycetes

<i>Fungus</i>	<i>Pigments</i>	<i>Ref.</i>
<b>Ustilaginales</b>		
<i>Ustilago scabiosae</i>	1, 2	65
<i>U. violacea</i>	3, 4	66, 67
<i>U. zeae</i>	1, 2, 3	65
<b>Uredinales</b>		
<i>Coleosporium euphrasiae</i>	5	2, 3
<i>C. pulsatilla</i>	5	68
<i>C. senecionis</i>	1, 2, (6?)	51
<i>Cronartium fusiforme</i>	1, 2, 3, 7, 8	69
<i>Gymnosporangium juniperinum</i>	5	70
<i>G. juniperi-virginianae</i>	1, 2	71
<i>Kunkelia nitens</i>	1, 2	72
<i>Melampsora aecidiodes</i>	5	70
<i>M. saliciapraeae</i>	5	68, 70
<i>Phragmidium violaceum</i>	5	2
<i>Puccinia coronata</i>	5	70
<i>P. coronifera</i>	1, 2, 3, 9, (10?)	70
<i>P. dispersa</i>	1, 2, 3	73
<i>P. graminis</i>	1, 2, 3, 9, (10?), 11	74
<i>Triphragmium ulmariae</i>	5	68, 70
<i>Uredo aecidioides</i>	5	2
<i>Uromyces alchemilliae</i>	5	2, 3
<b>Tremellales</b>		
<i>Calocera cosnea</i>	5	18
<i>C. palmata</i>	5	18
<i>C. viscosa</i>	1, 3, 10	53, 65
<i>Dacryomyces ellisii</i>	1, (2?), (3?), 10?)	75
<i>Dacryopinax spathularia</i>	1, 2, 10, 12	65
<i>D. stellatus</i>	1, 2, 9, (10?)	76
<i>Ditiola radicata</i>	5	2, 3
<i>Tremella mesenterica</i>	1	51

Key

- |                             |        |                            |        |
|-----------------------------|--------|----------------------------|--------|
| 1. $\beta$ -Carotene        | (1.13) | 7. $\zeta$ -Carotene       | (1.14) |
| 2. $\gamma$ -Carotene       | (1.38) | 8. $\beta$ -Zeacarotene    | (2.13) |
| 3. Lycopene                 | (1.11) | 9. Torulene                | (2.24) |
| 4. Neurosporene             | (2.14) | 10. $\beta$ -Cryptoxanthin | (1.60) |
| 5. Present but unidentified |        | 11. Phytoene               | (1.36) |
| 6. Rubixanthin              | (4.9)  | 12. Zeaxanthin             | (1.18) |

## 8.1.4 HYMENOMYCETES

A new pattern of carotenoid distribution appears in this Order, the synthesis of ketocarotenoids as major pigments. Canthaxanthin (4,4'-diketo- $\beta$ -carotene) was first isolated from *Cantharellus infundibilis*, but it is not present in all *Cantharellus* spp. so far examined and in some species neurosporene (2.14) predominates as if the final steps involving dehydrogenation to lycopene and subsequent cyclization were blocked. However, the recent report that in *C. infundibilis* 'neurosporene' is an epoxide [86] has not been

Table 8.4 Carotenoids in Hymenomycetes

Fungus	Pigments	Ref.
<b>Aphylophorales</b>		
<i>Cantharellus cibarius</i>	1, 2, 3	77
<i>C. cinnabarinus</i>	4, 5	78
<i>C. cornucopioides</i>	3, 6	65
<i>C. friesii</i>	1, 7	65
<i>C. ianthinoxanthus</i>	3, 6	79
<i>C. infundibilis</i>	3, 6, 8	17, 78
<i>C. lutescens</i>	1, 8, 9	53, 77, 80
<i>C. tubaeformis</i>	3, 6, 9	50, 77, 89
<i>Clavaria cardinalis</i>	10, 11	57
<i>C. helicoides</i>	10	65
<i>Laetiporus (Polyporus) sulphureus</i>	11	81
<i>Pencophora aurantiaca</i>	1, 2, 12, 13	65, 82
<i>P. hydnoides</i>	1, 12	65
<i>P. quercina</i>	1, 12, 13	65
<i>Pistillaria micans</i>	1, 2, 14	68
<b>Agaricales</b>		
<i>Clitocybe venustissima</i>	1, 2, 3	65
<i>Omphalia chrysophylla</i>	1, 2, 3, 15	65
<i>Phyllotopsis nidulans</i>	11, 12, 13, (16?)	65
<b>Nidulariales</b>		
<i>Sphaerobolus stellatus</i>	1, 2, 3, 4, 5, 13, 18	83-85

## Key

- |   |        |                                    |        |
|---|--------|------------------------------------|--------|
| 1. $\beta$ -Carotene                      | (1.13) | 10. 2,3-Didehydrophillipsiaxanthin |        |
| 2. $\gamma$ -Carotene                     | (1.38) | 11. Present but unidentified       |        |
| 3. Lycopene                               | (1.11) | 12. Astaxanthin                    | (1.68) |
| 4. Phytofluene                            | (1.37) | 13. Echinenone                     | (1.22) |
| 5. Canthaxanthin (4,4'-diketo-zeaxanthin) |        | 14. Torularhodin                   | (1.21) |
|   |        | 15. Torulene                       | (2.24) |
| 6. Neurosporene                           | (2.14) | 16. $\beta$ -Cryptoxanthin         | (1.60) |
| 7. Unidentified ketocarotenoid            |        | 17. Phytoene                       | (1.36) |
| 8. $\zeta$ -Carotene                      | (1.14) | 18. Zeaxanthin                     | (1.18) |
| 9. Dihydroxy- $\zeta$ -carotene           |        |                                    |        |



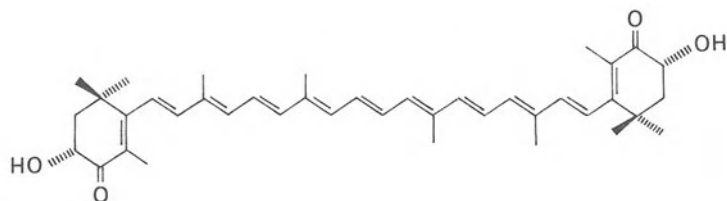
confirmed by mass spectrometry (G. Britton, unpublished). Other keto-carotenoids e.g. astaxanthin (1.68) have also been observed. The known carotenoid distribution in Hymenomycetes is outlined in Table 8.4.

### 8.1.5 GASTEROMYCETES

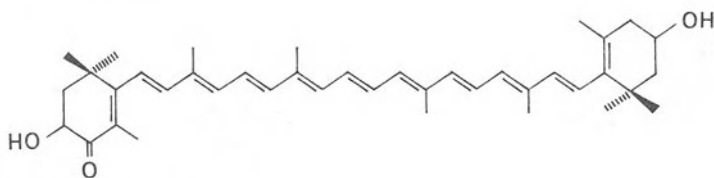
Very few members of this Order have been examined in detail and all are members of the Family Phallales. Unidentified pigments are present in *Clathrus cancellatus*, *Lysurus hexagonus*, *Mutinus bampipusinus* and *Pseudocolus* spp. [59]. Only hydrocarbons are present in *Anthurus aseriformis* [87, 88] (lycopene, neurosporene), *A. archeri* (lycopene with traces of the more saturated acyclic compounds) [89] and *Mutinus caninus* ( $\beta$ - and  $\gamma$ -carotenes) [90].

### 8.1.6 DEUTEROMYCETES

The distribution of carotenoids in Deuteromycetes is outlined in Table 8.5. Here is seen a homogeneous distribution consisting mainly of hydrocarbons including those with the  $\Delta^3$  double bond which are so characteristic of the red yeasts. Occasionally torularhodin and neurosporaxanthin, apocarotenoids



(8.6) 3*R*,3'*R*-Astaxanthin



(8.7) Phoenicoxanthin

which may be degradation products, are encountered. The presence of plectanixanthin (8.5), a pigment characteristic of the Discomycetes, in *Cryptococcus laurentii* is unexpected [94]. Perhaps the most exciting recent observation is the isolation of astaxanthin with chirality 3*R*,3'*R* (the opposite to that in lobster astaxanthin) from the red yeast *Phaffia rhodozyma* [106]. The two mating types of *Rhodospiridium* spp. synthesize the same carotenoids [108].

Table 8.5 Distribution of carotenoids in Deuteromycetes

<i>Fungus</i>	<i>Pigments</i>	<i>Ref.</i>
<i>Athrobotrys oligospora</i>	1, 2, 3(?), 4	91
<i>Cryptococcus albidus</i>	Present	92
<i>C. diffluens</i>	Present	92
<i>C. flavescens</i>	1, 4, 5	92
<i>C. laurentii</i>	1, 4, 5, 9	93, 94
<i>C. luteolus</i>	1, 4, 5	93
<i>Epicoccum andropogonis</i>	1, 4, 6, 7	95
<i>E. nigrum</i>	1, 4, 6, 7	96–98
<i>Fusarium aqueductuum</i>	1, (3?), 6, 8	99, 100
<i>F. orobonchus</i>	Present	101
<i>F. sambucinum</i>	1, 4, 6	100
<i>Phaffia rhodozyma</i>	10, 17, 18, 19, 20	105, 106
<i>Rhosporidium diobovatum</i>	1, 2, 4, 7, 8, 11, 12	102
<i>R. malvinellum</i> <sup>b</sup>	5, 8, 11, 12	102
<i>R. sphaerocarpum</i>	1, 2, 5, 11, 12	102
<i>R. toruloides</i>	(1?), 2, 4, 7, (8?), 11, 12	102
<i>Rhodotorula aurantiaca</i> <sup>a</sup>	1, 2, 4, 7, 8, 11, 12, 17	65, 95, 102, 104
<i>R. aurea</i>	1, 4	93
<i>R. flava</i>	1, 2, 4, 5	93
<i>R. glutinis</i>	1, 2, 4, 7, 8, 11, 12, 13, 14	65, 93, 107–111
<i>R. gracilis</i>	1, 2, 3, 4, 11, 12, 13, 14	112, 113
<i>R. infirmominata</i>	1, 2, 4	69
<i>R. mucilaginosa</i>	1, 2, 3, 4, 5, 7, 11, 12, 14, (?15)	114, 115
<i>R. minuta</i>	1, 2, 4, 7	93, 116
<i>R. pallida</i>	1, 3, 4	93
<i>R. peneaus</i>	1, 4, 5	93
<i>R. rubra</i>	1, 2, 4, 7, 8, 11, 12	93–110, 118–120
<i>R. sanniei</i>	1, 2, 3, 4, (?5)	65, 117
<i>R. spp.</i>	1, 3, 4, 7, 11, 12	102
<i>Sporidiobolus johnsonii</i>	1, 2, 4, 7	65, 121
<i>S. pararoseus</i>	2, 7	122
<i>Sporobolomyces roseus</i>	2, 4, 7	123, 124
<i>S. ruberrimus</i>	(?1), 2, 4, 7	125
<i>S. salmonicolor</i>	2, 4, 7	123
<i>S. shibatanus</i>	2, 8, 12, 13, 14	126
<i>S. spp.</i>	2, 4, (?7)	127
<i>Verticillium albo-atrum</i>	1, 2, 4, 5, 8, 11, 12, 13	128–131

## Key

1. $\gamma$ -Carotene	(1.38)	12. Phytofluene	(1.37)
2. Torulene	(2.24)	13. $\zeta$ -Carotene	(1.14)
3. Neurosporaxanthin	(8.1)	14. $\beta$ -Zeacarotene	(2.13)
4. $\beta$ -Carotene	(1.13)	15. 3,4-Didehydrolycopene	
5. Lycopene	(1.11)	16. 2-Hydroxyplectanixanthin	(1.22)
6. Rhodoxanthin	(4.10)	17. Echinenone	(1.22)
7. Torularhodin	(1.21)	18. 3-Hydroxyechinenone	
8. Neurosporene	(2.14)	19. Phoenicoxanthin	(8.7)
9. Plectanixanthin	(8.5)	20. 3-Hydroxy-3,4-dihydro- $\beta$ , $\psi$ - caroten-4-one	
10. Astaxanthin (3, <i>R</i> ,3' <i>R</i> isomer)	(8.6)		
11. Phytoene	(1.36)		

## Notes

<sup>a</sup> This is considered a mutant of the wild type.

<sup>b</sup> Rather unexpectedly reported to contain  $\alpha$ -carotene.

## 8.1.7 MYXOMYCETES

Only three members of this Order have been examined for carotenoids. Unidentified pigments are present in *Stemmitis ferruginea* [2] and *Lycogola flavofusum* [2, 3] whilst *L. epidendron* produces  $\beta$ - and  $\gamma$ -carotenes (1.13, 1.38), 3,4-didehydrolycopene, torulene (2.24) and neurosporaxanthin (8.1) [122, 123].

## 8.1.8 LOCALIZATION

Little recent work has been carried out on the localization of carotenoids in fungi but they are found in the mitochondria isolated from germinated spores of *Phycomyces blakesleeanus* [133]. In the C115 car-42-mad-107(–) mutant of *Phycomyces blakesleeanus*,  $\beta$ -carotene occurs both as oil droplets and in a particulate form [134]. Carotenoids are present in the cell wall of *B. trispora* although a slime mutant of *N. crassa* [134A] without a cell wall contains the usual complement of pigments [134B] (see Section 8.1.10).

## 8.1.9 NON-CAROTENOGENIC FUNGI

Unlike photosynthetic organisms, not all fungi synthesize carotenoids, and in Table 8.6 are listed those fungi which have been examined for carotenoids with negative results. Many more must, of course, exist.

Table 8.6 Fungi from which carotenoids appear to be absent [2, 3, 54, 65]

<i>Agaricus (Telamoria) armillatus</i>	<i>Cladonia coccifera</i>
<i>A. laceatus</i>	<i>Clavaria aurea</i>
<i>Allomyces cystogena</i>	<i>Clavaria corniculata</i>
<i>Alternaria solani</i>	<i>C. pallida</i>
<i>Amanita muscaria</i>	<i>C. pistillaris</i>
<i>A. pantherina</i>	<i>C. tunicula</i>
<i>Arthonia</i> spp.	<i>C. truncata</i>
<i>Ascobolus furfuraceus</i>	<i>Claviceps</i> spp.
<i>Auricularia auricula – judae</i>	<i>Clitocybe dealbata</i>
<i>Bachospora dryma</i>	<i>Cortinarius albo-violaceus</i>
<i>Bacidia muscorum</i>	<i>C. bulliardi</i>
<i>Biatora fungidula</i>	<i>C. caeruleus</i>
<i>Bilimbia melaena</i>	<i>C. evernii</i>
<i>Boletus luridus</i>	<i>C. praestans</i>
<i>B. scaber</i>	<i>C. traganus</i>
<i>Brettanomyces</i> spp.	<i>C. violaceus</i>
<i>Buellia</i> spp.	<i>Coryne sarcoides</i>
<i>Bulgaria inquinans</i>	<i>Cryptococcus neoformans</i>
<i>Candida</i> spp.	<i>Daedalea flavida</i>
<i>Cantharellus cibarius</i> var. <i>amethylteus</i>	<i>Discina perita</i>
<i>C. konradii</i>	<i>Disciotis venosa</i>
<i>Ciboria rufo-fusca</i>	<i>Femsjonia luteo-alba</i>

<i>Fusarium lycopersici</i>	<i>P. vesiculosa</i>
<i>Fusarium moniliforme</i>	<i>Phlebia radiata</i>
<i>Fusarium oxysporum</i> <sup>a</sup>	<i>Phragmidium violaceum</i>
<i>Ganoderma (Formes) lucidus</i>	<i>Pichia</i> spp.
<i>Gomphidius glutinosus</i>	<i>Polyporus grammoecephalus</i>
<i>G. viscidus</i>	<i>P. luzonensis</i>
<i>Guepinia helvelloides</i>	<i>P. rubidus</i>
<i>Gyromitra esculenta</i>	<i>P. zonalis</i>
<i>Helminthosporium sativum</i>	<i>Polystictus hirsutus</i>
<i>Helvella elastica</i>	<i>P. sanguineus</i>
<i>H. esculenta</i>	<i>P. versicolor</i>
<i>H. lacunosa</i>	<i>P. xanthopus</i>
<i>H. sulcata</i>	<i>Poromyces</i> spp.
<i>Hydnum ferrugineum</i>	<i>Pseudoplectania nigrella</i>
<i>H. repandum</i>	<i>Pullularia</i> spp.
<i>Hygrophorus coccineus</i>	<i>Pustularia catinus</i>
<i>H. punicens</i>	<i>Rhizoctonia solani</i>
<i>Hymenochate mougeotii</i>	<i>Rhizopogon rubescens</i>
<i>Kloeckera</i> spp.	<i>Russula alutacea</i>
<i>Lactarius deliciosus</i>	<i>R. aurata</i>
<i>Lactarius torminosus</i>	<i>R. emetica</i>
<i>Lecidea</i> spp.	<i>R. integra</i>
<i>Lenzites subferruginea</i>	<i>Rutstroemia echinophila</i>
<i>Morchella esculenta</i>	<i>Saccobolus violaceus</i>
<i>M. costata</i>	<i>Sarcogyme prunosa</i>
<i>Mycolachnea hemisphaerica</i>	<i>Sarcosphaera eximia</i>
<i>Neobulgaria pura</i>	<i>Scleroderma aurantium</i>
<i>Neogyromitra gigas</i>	<i>Sclerotinia tuberosa</i>
<i>Nephroma lusitanica</i>	<i>Sepultaria sumneriana</i>
<i>Neurophyllum</i> sp.	<i>Taphrina communis</i>
<i>Oidium violaceum</i>	<i>T. deformans</i>
<i>Omphalia abiegna</i>	<i>Thalloidima candidum</i>
<i>Otidea alutacea</i>	<i>Thelephorus</i> spp.
<i>O. concinna</i>	<i>Thielavia terricola</i>
<i>O. onotica</i>	<i>Torulopsis lipofera</i>
<i>O. umbrina</i>	<i>T. luteola</i>
<i>Paxillus atrotomentosus</i>	<i>T. pulcherrima</i>
<i>Paxina acetabulum</i>	<i>Trametes persooni</i>
<i>Penicillium clavariaeformis</i>	<i>T. versatilis</i>
<i>Peziza badia</i>	<i>Trichophaea confusa</i>
<i>P. badiocfusa</i>	<i>Trichosporon</i> spp.
<i>P. cerea</i>	<i>Trigonopsis</i> spp.
<i>P. echinospora</i>	<i>Urnula pouchetii</i>
<i>P. sanguinea</i>	<i>Verpa conica</i>
<i>P. succosa</i>	<i>Zygosaccharomyces</i> spp.

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<sup>a</sup> Carotenoids synthesized after photoinduction (see Table 8.13).

## 8.1.10 MUTANTS

A series of *Neurospora crassa* mutants obtained some years ago divided themselves into four main groups in which synthesis of the following were blocked (i) acidic carotenoids such as neurosporaxanthin (8.1); (ii) most xanthophylls, some carotenes but not phytoene (1.36); (iii) all polyenes except phytoene and (iv) all polyenes including phytoene [135]. In four crosses between a wild type and an albino *N. crassa*, *CI* and *Ci* contained the same pigments as the wild type [*C* is the major colour gene and *I* the intensifier], but in reduced amounts: *ci* contained no carotenoids and *cI* only traces [136]; albino mutants of *N. crassa* have been obtained which when cultured together form a carotenoid-producing mycelium in which the haploid nuclei from both strains occur side by side in the cytoplasm but do not fuse; no carotenoids are synthesized by either mutant growing singly or in the presence of a mycelial extract of the other [137]. A mutant *ylo-1* of *N. crassa* contained phytofluene (1.37),  $\zeta$ -carotene (1.14),  $\beta$ -carotene (1.13),  $\gamma$ -carotene (1.38), neurosporene (2.14) and lycopene (1.11) and had obviously been blocked in the desaturation of lycopene at C-3: albino mutant *al-1* contained large amounts of phytoene and only traces of other polyenes [47]. The carotenoid-less mutants of *N. crassa* lack a single protein which is present in the wild type. This suggests that synthesis takes place on a single protein complex [138]. A slime mutant of *N. crassa* which lacks a cell wall contains the same carotenoids as the wild type [134B]. Orange and yellow mutants of *Ustilago violacea* contain  $\gamma$ -carotene and  $\beta$ -carotene, respectively, whilst the pink wild type produces mainly neurosporene and lycopene [66].

Various mutants and strains of *Rhodotorula* spp. have been described [93, 103, 114, 119]; in particular it is reported that one mutant of *R. glutinis* produces twice as much  $\beta$ -carotene as the native strain [109].

Certain naturally occurring mutants of *Phycomyces blakesleeana* all produce  $\beta$ -carotene but in varying amounts [29]. A number of artificial mutants of this organism have been isolated [139] and they fall into three main categories: (i) those which still synthesize  $\beta$ -carotene in greater or lesser amounts than the wild type: (ii) those which produce mainly phytoene and (iii) those producing mainly lycopene. Mutants which differ from the wild type in their ability to synthesize carotenoids are designated *car*, whilst those strains which differ from the wild type in their phototrophic response are designated *mad*. For example, Table 8.7 shows that *mad-107(-)* synthesizes about five times as much  $\beta$ -carotene as the wild strain; *car-10(-)* synthesizes mainly phytoene (about twice as much as the wild strain) and *car* R21(-) mainly lycopene and phytoene (total about five to ten times normal) [139, 140]. Complementation studies showed that each of the mutant types corresponded to three mutants in a single cistron, termed *car* R, *car* B and *car* A, respectively [141–143]. Genes *car* B and *car* R are structural genes for phytoene dehydrogenation and cyclization of lycopene, respectively. Gene

*car A* is defined by mutants which do not synthesize significant amounts of carotenoids because of low levels of  $\beta$ -carotene required for negative control [332]. An additional gene *car S*, the product of which is involved in end-product regulation of carotenogenesis, it is probably a diffusible, cytoplasmic negative regulator. Some intense yellow mutant strains synthesize some 50–70 times as much  $\beta$ -carotene as native strains [144].

Mutation *car-102* renders constitutive the stimulation of  $\beta$ -carotene synthesis which in the wild type depends on the presence of retinol [145] (see also Section 8.2.13). If the stimulatory actions of *sex*, *car S* mutation and *car-102* mutation are combined in one heterokaryon stabilized by the presence of balanced lethal mutation, then the resulting strain accumulates over 500 times more carotene than normal strains under normal conditions, that is 25 mg/g dry matter [145].

Further analysis of these mutants is still in progress but the suggestion is that *car A* and *car B* represent separate segments of a single gene, and that *car A* mutations affect one segment and *car B* mutations the other [145]. Many interesting developments are expected in this field in the near future.

Table 8.7 Carotenoids content of wild type and mutants of *Phycomyces blakesleeanus*

	Wild type	<i>mad-10</i> (–)	<i>car-10</i> (–)	<i>car R 21</i> (–)
		$\mu\text{g polyene/g dry wt. sporangiophores}$		
Phytoene (1.36)	—	—	400	70
Phytofluene (1.37)	—	—	2	20
$\zeta$ -Carotene (1.14)	—	—	0.1	10
Neurosporene (2.14)	—	—	0.05	0.5
$\beta$ -Zeacarotene (2.13)	—	—	0.11	0.5
Lycopene (1.11)	—	—	0.05	700
$\gamma$ -Carotene (1.38)	—	—	0.05	0.5
$\beta$ -Carotene (1.13)	50	220	0.1?	0.4

A series of coloured mutants of *Verticillium albo-atrum* synthesizes acyclic polyenes including lycopene (1.11) as well as neurosporaxanthin (8.1), although the wild type is colourless and does not synthesize carotenoids [129, 130]. Native *Fusarium aquaeductuum* synthesizes carotenoids only after photoinduction (see Section 8.2.13(b)) but a number of mutants have been described which lack photoregulation and will synthesize carotenoids in the dark [1, 46]. A series of mutants of *Epicoccum nigrum* mostly produce more carotenoids than the wild strain and the following polyenes have been detected: phytoene (1.36), lycopene (1.11), 3,4-didehydrolycopene, 3,4,3',4'-tetrahydrolycopene,  $\beta$ -cryptoxanthin (1.60), torulene (2.24) and traces of what was identified as eschscholtzanthin (1.25) [147].

## 8.2 GENERAL FACTORS CONTROLLING SYNTHESIS

### 8.2.1 PATTERN OF SYNTHESIS

Three main phases of carotenoid synthesis are observed in *Rhodotorula rubra*: (i) an initial period of active synthesis leading to maximum concentration; (ii) an intermediate period of persistence during which no major changes, either qualitative or quantitative, occur; (iii) a final period during which the pigments disappear [148]. Somewhat similar patterns are observed in *Rhodotorula gracilis* [149], static cultures of *Phycomyces blakesleeanus* [150], *Penicillium sclerotiorum* NRRL 2074 [40] and in *Epicoccum nigrum* [96, 97]. The pattern is less marked in shake cultures of *P. blakesleeanus* but still obvious [151]. In wild type *P. blakesleeanus* the rate of carotenoid synthesis during the early stages of phase (i) in static culture is slow, the major synthesis occurring after growth is complete [150]. Thus if only sufficient glucose is present in the medium for maximum growth (in the experiments under discussion 1.5%) then the synthesis of  $\beta$ -carotene is limited. With excess glucose then synthesis is markedly stimulated. In other words a medium with a low carbon to nitrogen (C:N) ratio will be much less effective than one with a high C:N ratio. This conclusion holds for a number of other fungi such as *Sporobolomyces roseus* [152], *Rhodotorula sarniei* [153], *Rhodotorula* sp. [154] and *Blakeslea trispora* [155]; in the last organism a C:N ratio of 55 when 3% glycerol is the carbon source is optimal for carotenogenesis.

An exception to this general pattern is *Rhodotorula gracilis* growing on a molasses/(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> medium when alteration of the C:N ratio does not significantly change the ability of the yeast to synthesize carotenoids [156, 157].

In some cases marked qualitative changes occur during active carotenogenesis. Young (red) cultures of *Rhizophlyctis rosea* contain mainly lycopene (1.11); older cultures turn yellow owing to the massive synthesis of  $\gamma$ -carotene (1.38) which masks the red colour of the lycopene which is still present [6]. Similar changes are observed in *Epicoccum nigrum* [158] and the red mutants *car 9*, *car 10*, *car 13* of *P. blakesleeanus* and the heterokaryon C2\*C9 [145]. However, in the heterokaryon C2\*C9 the present evidence is somewhat contradictory; according to de la Guardia *et al.* [159] lycopene concentration remains relatively constant over an incubation period of 48–169 h, whilst that of  $\beta$ -carotene increases some ten times. Hsu *et al.* [151], on the other hand, find that although the pattern of  $\beta$ -carotene synthesis increases as first reported, lycopene is higher after 48 h than later (Table 8.8).

In the final period of incubation of carotenogenic fungi the pigments gradually disappear and mutatochrome (5,8-epoxy- $\beta$ -carotene) an intermediate in the oxidative decomposition of  $\beta$ -carotene, has been found in old *P. blakesleeanus* cultures [160]; this may easily be formed non-enzymically

Table 8.8 Carotenoid levels at various stages of growth of the red *Phycomyces* mutant *car-B* [167]

Age of culture (h)	48.5		96		169	
	$\mu\text{g/g}$ dry wt.	% of total	$\mu\text{g/g}$ dry wt.	% of total	$\mu\text{g/g}$ dry wt.	% of total
Phytofluene (1.36)	52	12	36	4.0	59	6.0
$\zeta$ -Carotene (1.14)	12	3	19	2.0	7	0.7
Neurosporene (2.14)	9	2	13	1.5	19	2.0
Lycopene (1.11)	330	77	260	27.7	224	23.6
$\gamma$ -Carotene (1.38)	18	4	70	7.5	80	8.4
$\beta$ -Carotene (1.13)	9	2	238	57	556	58.5
$\beta$ -Zeacarotene (2.13)	—	—	3	0.3	8	0.8
Total	430		939		975	

by the isomerization of enzymically formed 5,6-epoxy- $\beta$ -carotene under the strongly acid conditions found in old cultures of *P. blakesleeana*.

## 8.2.2 CARBON SOURCES

Variations in the carbohydrate source in the culture medium affect carotenogenesis differently in different fungi. In *Rhodotorula sarniei*, for example, glycerol is the most effective single source although a mixture of lactose and glucose is equally good; glucose alone is non-carotenogenic but when colourless cells produced on a glucose/gelatin medium are transferred to a suitable medium they will synthesize pigments [161]. Complex sources are also suitable for carotenogenesis in red yeasts, for example molasses are effective for *R. gracilis* [156], *R. glutinis* [162] and *Sporobolomyces pararoseus* [163], whey for *R. lactis* [164], hydrolysed peat for *Sporobolomyces pararoseus* [102, 165] and other red yeasts [166], paraffin for *R. glutinis*, *R. graminis* and *Sporobolomyces* spp. [167] and for *Blakeslea trispora*, *n*-decane and *n*-pentadecane [168, 169] as well as sunflower oil [155].

In *Phycomyces blakesleeana* maltose and glucose are equally carotenogenic whilst xylose and fructose, which are equal to maltose and glucose in sustaining growth, are much less carotenogenic. On the other hand *P. blakesleeana* will not grow on lactose or glycerol as the sole carbon source [25]. Xylose and fructose are the best carbohydrates for carotene production in *Neurospora sitophila* [170] and hexoses also stimulate synthesis by *Sporobolomyces pararoseus* and a *Candida* sp. [171].

Growth and carotenogenesis are both sparse in *P. blakesleeana* growing on lactate as sole carbon source but carotenogenesis is specifically stimulated by addition of acetate up to a concentration of 1.12%; at higher concentrations pigment production rapidly falls off [172]. Acetate also increases carotenogenesis in *Rhodotorula sarniei* if the basic medium is low in nitrogen



[173]. Wild type *Blastocladiella* does not normally synthesize carotenoids but in the presence of high concentration of  $\text{HCO}_3^-$  it synthesizes  $\gamma$ -carotene (1.38). Similarly a mutant deficient in  $\alpha$ -oxoglutaric oxidase also makes  $\gamma$ -carotene and it has been suggested that in the wild type high  $\text{HCO}_3^-$  levels inhibit the tricarboxylic acid cycle and channel metabolites, which are normally dealt with via the tricarboxylic acid cycle, into carotenoid synthesis [174].

### 8.2.3 NITROGEN SOURCES

If asparagine is replaced in the basic culture medium by either valine or leucine then the synthesis of  $\beta$ -carotene by *Phycomyces blakesleeanus* is greatly stimulated [175, 176]. This effect is considered in detail in Chapter 2, but it is known to be due to the formation of  $\beta$ -hydroxy- $\beta$ -methylglutaryl-CoA (HMG-CoA) a key intermediate in isoprenoid synthesis, from the two amino acids (Chapter 2). HMG-CoA formation must in this case be rate-limiting for carotenogenesis under the conditions employed. The stimulatory effect of leucine is apparent in *Rhodotorula gracilis* and increased synthesis is also elicited by addition of glutamic acid [177] but the reason for this is not known. However, *R. aurantiaca* synthesizes less carotenoid if grown in the presence of sulphur-containing amino acids than in their absence [178]; there is also a qualitative effect, the addition of sulphur-containing amino acids causes the ratio of  $\gamma$ -carotene: $\beta$ -carotene:torulene:torularhodin to change from 1:2:17:30 in the standard medium to 1:1:8:2 [135].

Labelled glycine [179] and formate [180] are effectively incorporated into  $\beta$ -carotene by *P. blakesleeanus*. It is suggested that this is due to the effective incorporation of these substrates into acetyl-CoA by the following pathway: formate  $\rightarrow$  glycine  $\rightarrow$  serine  $\rightarrow$  pyruvate  $\rightarrow$  acetyl-CoA.

### 8.2.4 pH

The optimal pH for carotenogenesis in *Rhodotorula gracilis* is 5.0–5.6 [181] and for *Rhodotorula lactis* growing on whey it is 7.0 [182]. The pigments in *R. sanniei* are qualitatively unchanged when the initial pH of the culture medium is varied from 5.2 to 7.6 [183]. A fuller investigation on a *Rhodotorula* sp. indicated maximal carotene synthesis when the initial pH of 4.0–4.7 was allowed to drop to 3.1–3.3 after 72 h and rise to a final value of 5.7–5.9 [184]. On the standard glucose/asparagine medium the pH drops from an initial 6.2 to 2.6–3.0 during growth of *Phycomyces blakesleeanus*: if the drop in pH is prevented by buffering the medium, growth is unaffected although carotenogenesis is almost completely inhibited. Similarly, washed mats of *P. blakesleeanus* metabolizing glucose will produce  $\beta$ -carotene only on an unbuffered medium [185].

If asparagine is replaced by ammonium sulphate in the basic medium for

*P. blakesleeanus* then growth and carotenogenesis are reduced; however, addition of any member of the tricarboxylic acid cycle at a level 0.02 M restores growth to normal but only acetate and succinate restore normal carotenogenesis [172, 185, 187]. The change in the final pH of the culture medium observed with different tricarboxylic acids may account for the differential effect on carotenogenesis.

#### 8.2.5 TEMPERATURE

The pigments produced by *Rhodotorula sarniei* are the same over the temperature range 14–28°C [183] and the same applies to *R. rubra* and *R. peneaus* (5–25°C) [93] as well as to *Phycomyces blakesleeanus* (5–25°C) [187]. In general lower temperatures result in less pigment production; for example the optimum temperature for carotenogenesis in *Neurospora sitophila* is 28–30°C [170]. In one red yeast, *R. gracilis*, temperature changes bring about marked qualitative changes although unanimity does not exist on the nature of these changes. Two reports indicate the production of yellow mycelia at low temperatures, with  $\alpha$ - and  $\beta$ -carotenes predominating, and red mycelia at high temperatures with torulene and torularhodin predominating [93, 188]; on the other hand it is also claimed that temperature has no effect on  $\beta$ -carotene synthesis and that more torularhodin is produced at 17°C than at 27°C or 37°C [113]. Qualitative changes also occur in *R. glutinis* [107].

#### 8.2.6 TRACE ELEMENTS

In *Neurospora sitophila* the presence of Co, Cu, Mn, or Zn increased  $\beta$ -carotene (1.13) production, but only Mn increased the synthesis of lycopene (1.11), neurosporene (2.14),  $\zeta$ -carotene (1.14) and phytofluene (1.37) [189]. When *Rhodotorula gracilis* is grown on molasses carotenogenesis is improved by the addition of trace elements [175]. Fluoride added to the medium increases carotenogenesis in *P. blakesleeanus* by five times. The effect is possibly due to the inhibition of phosphatases which could dephosphorylate key intermediates such as isopentenyl pyrophosphate [190].

#### 8.2.7 PRESSURE

It is reported that high pressure (800 atm) has the same effect on carotenogenesis in *B. trispora* as the inhibitor diphenylamine (see Section 8.2.10) [191].

#### 8.2.8 GROWTH FACTORS

Low thiamine levels tend to reduce carotenogenesis by *P. blakesleeanus* grown on a glucose-based medium but the effect is not dramatic. On an acetate-based medium the addition of yeast extract is stimulatory but the factor

responsible has not yet been identified [172]. A slight but significant stimulation was observed in *Mucor hiemalis* in the presence of pantothenic acid [192]. Riboflavin in concentrations above 0.005% inhibits carotene synthesis in *P. blakesleeanus* whilst lower concentrations tend to be stimulatory although this may be basically due to a stimulation in growth rate [193]. Lack of biotin stimulates pigmentation in *Rhodotorula* spp. and *Sporobolomyces* spp. [194], which may be the result of channelling acetyl-CoA away from fatty acid synthesis by reducing its conversion into malonyl-CoA, a biotin-dependent step. However, biotin stimulates carotenogenesis in *P. blakesleeanus* and *B. trispora*, whilst avidin, a biotin inhibitor, reduces synthesis by 50–70% [195]. High levels of *p*-aminobenzoic acid completely suppressed carotenoid synthesis in *Neurospora crassa* [196]. Vitamin A greatly stimulates carotenogenesis in wild type and mutant strains of *P. blakesleeanus* [197]; this effect is discussed in detail in Section 8.2.13.

### 8.2.9 OXYGEN

Strong aeration stimulates carotenogenesis in *Rhodotorula gracilis* [198, 199].

### 8.2.10 INHIBITORS

The most fully investigated inhibitor of carotenogenesis in fungi is diphenyl-

Table 8.9 Fungi in which carotenogenesis is inhibited by diphenylamine with simultaneous accumulation of phytoene

Organism	Ref.
<i>Allomyces</i> spp.	8, 196
<i>Blakeslea trispora</i>	14, 200, 201
<i>Epicoccum nigrum</i>	202
<i>Fusarium</i> spp.	203
<i>Leptosphaeria michotii</i>	204
<i>Neurospora crassa</i>	8
<i>Phycomyces blakesleeanus</i> (wild type)	25, 205, 206
<i>P. blakesleeanus</i> (mutants)	140, 206
<i>Pilobolus</i> spp.	207
<i>Rhodosporidium</i> spp.	102
<i>Rhodotorula gracilis</i>	208, 209
<i>R. mucilaginosa</i>	210
<i>Sporobolomyces shibatanus</i>	211
<i>Verticillium albo-atrum</i>	130, 212
<i>V. agaricinum</i> <sup>a</sup>	213

Note

<sup>a</sup>No accumulation of phytoene observed.

amine (DPA). The fungi in which it inhibits the synthesis of the normal carotenoids with the accumulation of massive amounts of phytoene and small amounts of other partly saturated carotenes are listed in Table 8.9. It is also an effective inhibitor in some photosynthetic and non-photosynthetic bacteria but not in algae. Its action is discussed in Chapter 2. Inhibition of dehydrogenation steps is also observed with benzophenone in *Mucor hiemalis* [214]. A new series of inhibitors have been described which prevent synthesis of  $\beta$ -carotene and related products and allow the accumulation of lycopene; that is, they inhibit the cyclization of acyclic carotenoids. The first reports of this type of inhibition concerned the effect a number of imidazole and pyridine derivatives on carotenogenesis by *Blakeslea trispora* [140, 215]. Many interesting observations emerge from the consideration of the data provided (Table 8.10); not all derivatives are active [e.g. 5-(2-hydroxyethyl imidazole)] whilst some, for example 3-formylpyridine, stimulate both  $\beta$ -carotene and lycopene synthesis. On the other hand the closely related 2-formylpyridine is without any effect. Nicotine which is inhibitory in *P. blakesleeanus* (native [216, 217] and mutant strains [140]) and *Verticillium agaricinum* has also been examined in some detail [213]. The result of nicotine inhibition is a dramatic drop in the synthesis of cyclic carotenoids and the equally dramatic

Table 8.10 The effect of some imidazole and pyridine derivatives on carotenogenesis by *Blakeslea trispora* [215]

Inhibitor	Concentration (g/l)	Carotene production (mg/l medium)	
		Lycopene	$\beta$ -Carotene
None		40	750–820
Imidazole	1	750	20
2-Methylimidazole	0.01	855	180
5-(2-Hydroxyethyl)-imidazole	1	50	795
1-Ethyl-2-methylimidazole	1	865	20
Pyridine	3	760	25
2-Aminopyridine	0.1	805	20
3-Formylpyridine	2	560	780
2-Formylpyridine	0.5	55	750

accumulation of lycopene. This is illustrated in Table 8.11 with results obtained with a mutant strain of *P. blakesleeanus*. In this case removal of the inhibitor from the culture does not allow the cyclization of lycopene to proceed although this does occur in bacteria (see Chapter 2). The herbicide CPTA [2-(4-chlorophenylthio)triethylamine hydrochloride] has a similar effect on cyclization in *P. blakesleeanus* [218] and *Blakeslea trispora* [219, 220] as do a number of other amines, namely 4-[ $\beta$ -(diethylamino)-ethoxy]-benzaldehyde, 4-[ $\beta$ -(diethylamino)-ethoxy]-acetophenone hydrochloride, 4-[ $\beta$ -(diethylamino)-ethoxy]-benzophenone hydrochloride, triethylamine hydrochloride,

Table 8.11 Effect of nicotine on  $\beta$ -carotene and lycopene production in mutant *mad-107*(–) of *Phycomyces blakesleeanus* [140]

Nicotine conc. (mM)	Carotene conc. ( $\mu\text{g/g}$ dry wt.)	
	$\beta$ -Carotene	Lycopene
0	3780	0
1.0	3585	36
5.0	2120	1230
7.0	5	2660

$\alpha$ -diethylaminopropiophenone hydrochloride and tributylamine hydrochloride [220]. In *P. blakesleeanus* mutants cultured in the presence of CPTA lycopene accumulates at the expense of  $\beta$ -carotene [221] and in the case of *Rhodotorula glutinus* and *R. rubra*, lycopene accumulates at the expense of  $\beta$ -carotene, torulene (2.24) and torularhodin (1.21) [111]. CPTA is said to have no significant effect on *Verticillium agaricinum* [213, 222] but some quantitative changes are apparent on close study of the data presented. Other inhibitors of fungal carotenogenesis include methyl heptenone [213, 223, 224], 2-hydroxybiphenyl [210], 4-aminopyridine [200, 201], citral [225], dimethylsulphoxide [206] and  $\beta$ -ionone [213, 226] which, however, is usually stimulatory (Section 8.2.13).

### 8.2.11 ANTIBIOTICS

A number of antibiotics have been tested for their effects on carotene synthesis in fungi and the observations are summarized in Table 8.12.

### 8.2.12 STIMULATORS

Surfactants of various types stimulate carotenogenesis in *Blakeslea trispora* [12, 140, 168, 231] and *Sporobolomyces roseus* [171].

### 8.2.13 BIOINDUCTION OF CAROTENOGENESIS

#### (a) *Trisporic acid* and related compounds

When (+) and (–) strains of the heterothallic fungus *Choanephora cucurbitarium* [15] are cultured together, some twenty times more carotene is formed than in either the (+) or (–) strain cultured separately; such sex-stimulated carotenogenesis occurs in both mating types separated by a cellophane membrane. The same phenomenon has been observed with (+) and (–) strains of *Blakeslea trispora* and *Phycomyces blakesleeanus* [12, 15–17, 232–241, 316]. The stimulation makes industrial production of  $\beta$ -carotene

Table 8.12 The effect of some antibiotics on carotenogenesis in fungi<sup>a</sup>

Antibiotic	Fungus	Effect	Ref.
Actinomycin D	<i>Verticillium</i>	Inhibits	227
	<i>Neurospora</i>	Inhibits photoinduced carotenogenesis	228
Aureomycin	<i>Phycomyces</i>	Inhibits non-specifically	168
Biomycin	<i>Rhodotorula</i>	No effect	198
Cycloheximide	<i>Allomyces</i>	Stimulates	229
	<i>Blastocladiella</i>	Stimulates	174
	<i>Phycomyces</i>	Inhibits	225
	<i>Verticillium</i>	Inhibits	227
Penicillin	<i>Phycomyces</i>	No effect	168
	<i>Rhodotorula</i>	Inhibits	198
Isoniazid	<i>Verticillium</i>	Inhibits	230
Puromycin	<i>Verticillium</i>	Inhibits	168
Streptomycin	<i>Phycomyces</i>	No effect on $\text{NH}_4 \text{NO}_3$	168
		Inhibits on asparagine	227

#### Note

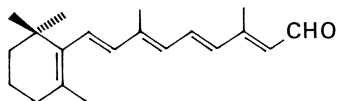
<sup>a</sup> See Section 8.2.13 for the effect of antibiotics on induced carotenogenesis.

feasible under appropriate conditions [233]. The biostimulator has been isolated from mated cultures of *B. trispora* and consists of a series of carboxylic acids of which trisporic acid C (2.10) is the major component [237–239]. Trisporic acid B [238], and ‘Compound 3’ [239] are probably identical. The primary biological function of trisporic acid or a close metabolite thereof is as a sex hormone controlling zygomorph formation in the Mucorales [242–247]. It also controls gametogenesis in *Phycomycetes* [240].

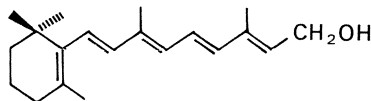
Trisporic acid stimulates carotenogenesis in the (–) strain but not in the (+) strain of *B. trispora* [14, 247, 248]. However, it appears to be the (+) strain which synthesizes the trisporic acids because when either the (+) or (–) strain is co-cultured with the homothallic *Zygorhynchus moelleri*, trisporic acid is formed only in the presence of the (+) strain [247]. Physical contact between the (+) and (–) strains is probably not necessary for trisporic acid synthesis which is stimulated by a diffusible ‘progamone’ formed in and released from the (–) strain [239]. Trisporic acid does not stimulate carotenogenesis in other mucors, however, but it does in strains of *B. trispora* which do not produce endogenous trisporic acid [236, 249].

Experiments with [<sup>14</sup>C] trisporic acid showed that it is itself not incorporated into the  $\beta$ -carotene, whose synthesis it stimulates [236], and its effect is inhibited by cycloheximide, a potent inhibitor of protein synthesis by cytoplasmic ribosomes [250, 251], but only partly by actinomycin D [251]. Furthermore in the presence of diphenylamine, trisporic acid stimulates the synthesis of phytoene and not  $\beta$ -carotene [14]. All these observations indicate

that trisporic acid probably functions by derepressing the synthesis of an enzyme concerned with an enzymic step prior to phytoene synthesis and that the control is at the translation phase of protein (enzyme) synthesis. There is evidence that  $\beta$ -carotene is itself the precursor of trisporic acid which could be formed via retinal (8.8) and retinol (vitamin A) (8.9) [252, 253]; if this is so then the system represents a positive feed-back control. The nature of the immediate precursors (prohormones) of the trisporic acids has recently been reviewed [254].



(8.8) Retinal



(8.9) Vitamin A (retinol)

$\beta$ -Ionone has a similar stimulatory effect on both mating types of *Phycomyces blakesleeanus* [255–258] and on heterothallic but not homothallic cultures of *Blakeslea trispora* [13, 140, 179, 259–262]. Other terpenes [263] and derivatives of  $\beta$ -ionone [215] are also stimulatory and the action of  $\beta$ -ionone derivatives and the carotenogenic amines are cumulative [215]. Vitamin A itself is particularly active in native and mutant strains of *P. blakesleeanus* [258] and *B. trispora* [259]; its effect, as is that of  $\beta$ -ionone, is inhibited by cycloheximide [258]. The failure of chloramphenicol to inhibit the  $\beta$ -ionone effect [260] is almost certainly due to the fact that this antibiotic does not inhibit cycloplasmic protein synthesis in eukaryotes but is specific for chloroplast protein synthesis. As  $\beta$ -ionone also stimulates protein and RNA synthesis in *B. trispora* it is suggested that it functions by stimulating *de novo* protein synthesis at the translational level [262] and this is supported by the finding that  $\beta$ -ionone and trisporic acid are competitive [264] and that the actions of both are inhibited by cycloheximide [259]. Citral inhibits carotene synthesis in *Rhodotorula gracilis* but stimulates phytoene synthesis [265], an effect akin to that of diphenylamine. A quite different chemical, ammonium perchlorate (20–2000  $\mu$ g/ml), is said to stimulate  $\beta$ -carotene (1.13) synthesis to a much greater extent than torularhodin (1.21) synthesis in *R. gracilis* [266]. Cyclic AMP ( $10^{-4}$  M) stimulates carotene formation in *N. crassa* when added to an  $\text{NH}_4^+$  glucose medium before inoculation [267], and succinimide stimulates in *B. trispora* [140].

### (b) Light

*General.* Light frequently stimulates carotenogenesis in fungi which normally form reasonably impressive amounts in the dark; for example, in *Phycomyces blakesleeanus* [25, 223, 268, 269, 326], *Penicillium oxysporum* [40], *Rhodotorula rubra* [93], *R. peneus* [93], *R. gracilis* [107], *Epicoccum nigrum* (at 28°C) [270], *Sporidiobolus johnsonii* [271], *Neurospora crassa* [140, 272] and *Leptosphaeria michotii* [42, 273]. The effect on *P. blakesleeanus* and *P.*

*oxysporum* is merely quantitative; qualitative changes are observed in *R. gracilis* and *S. johnsonii*, but in the opposite direction; in *R. gracilis* carotene synthesis is stimulated in the light whilst in *S. johnsonii* torularhodin synthesis is stimulated. In the carotene fraction of *Neurospora crassa* the neurosporene (2.14) level tends to fall and those of  $\gamma$ - and  $\beta$ -carotenes (1.38, 1.13) to rise in illuminated cultures compared with non-illuminated cultures [40]. The opposite effect, reduction of carotenogenesis on illumination, is observed in *Blastocladiella emersonii* [274], *Choanephora cucurbitarium* [275], and *Blakeslea trispora* [276]. In *Epicoccum nigrum* high intensity illumination (2500 lx) at 24°C reduces carotenogenesis, although at 24°C stimulation is observed.

**Photoinduction.** In some other fungi in which carotenogenesis in the dark is either nil or very slight, considerable stimulation is achieved by photoinduction which involves a short simultaneous exposure to oxygen and light followed by a return to darkness. Fungi known to exhibit this phenomenon are listed in Table 8.13.

Table 8.13 Fungi in which photoinduction of carotenogenesis has been reported

Organism	Ref.
<i>Aspergillus giganteus</i> m. <i>alba</i>	277
<i>Cephalosporium diospyri</i>	278, 279
<i>Dacrospina</i> <i>spathularia</i>	280
<i>Fusarium aquaeductum</i>	131, 281–287
<i>F. coeruleum</i>	288
<i>F. oxysporum</i>	289
<i>Leptosphaeria michotii</i>	184
<i>Neurospora crassa</i>	134, 282, 290–294
<i>N. sitophila</i>	170
<i>Pyronema confluens</i>	64
<i>Sphaerobolus stellatus</i>	295
<i>Syzygites megalocarpus</i>	327
<i>Verticillium</i> spp.	194, 296

The action spectrum for photoinduction in *N. crassa* and *F. aquaeductum* resembles the absorption spectrum of a flavoprotein (Fig. 8.1) [291, 283, 298] and is similar to the action spectrum for phototropism in *Avena* coleoptiles and *Phycomyces* sporangiophores (see Chapter 3); the blue light which photoactivates carotenogenesis also stimulates photoreduction of a *b*-type cytochrome [297, 328–330]. Recent measurements of photoinduction in *N. crassa* suggest that the photoreceptor may be  $\beta$ -carotene itself and it has been suggested that the pigment functions as both photoreceptor and regulator [298]. The case for a flavoprotein as the photoreceptor has been discussed in detail [299, 300]. Red light can induce carotenogenesis in the presence of redox dyes, such as methylene blue and toluidine blue, which



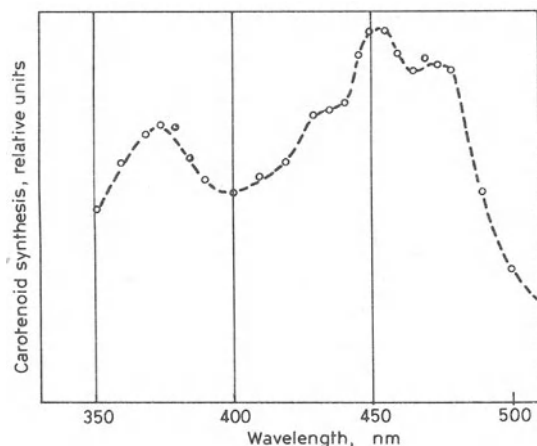


Fig. 8.1. Action spectrum for photoinduced carotenoid synthesis in *Fusarium aquaeductuum* ○---○---○. (Redrawn from [300].)

absorb in the active region of the spectrum and presumably act as artificial photoreceptors [301].

After photoinduction a lag period of, for example, 4 h in *F. aquaeductuum* [283] and 40–90 min in *N. crassa* [282], occurs. At low temperatures (6–10°) the lag period increases to about 6 h in both *N. crassa* [301] and *F. aquaeductuum* [302]. This lag period suggests that synthesis of new protein is associated with photoinduction and photo-stimulated synthesis of protein has been demonstrated in *Verticillium agaricinum* [303]; furthermore a new protein is observed in *N. crassa* after illumination [138, 304].

If illuminated aerobic cells are transferred to darkness under nitrogen then the lag phase for carotenogenesis is eliminated when oxygen replaces nitrogen, indicating that the necessary enzymes have been synthesized during the dark anaerobic phase [305].

If it is accepted that enzyme synthesis is a result of photoinduction then two questions arise: (i) is the control at the transcription or translation stage in protein synthesis? and (ii) which enzyme or enzymes are photoinduced? Antinomycin D, an inhibitor of transcription, partly inhibits photoinduced carotenogenesis in *V. agaricinum* [212] and *N. crassa* [213, 332], but in *Fusarium aquaeductuum* the block by distamycin A, another inhibitor of transcription, is complete [300]. Regulation at the transcription stage implies a *de novo* synthesis of specific messenger RNAs on photoinduction and this has recently been demonstrated [300, 306, 307].

With regard to the nature of the enzyme(s) under photocontrol it has been shown that in cell-free preparations of a *Mycobacterium*, phytoene synthetase is one of the enzymes involved (see also Chapter 9) [308]. However, phytoene is present to a limited extent in dark-grown fungi in which photo-induction operates [212, 290, 308] and this suggests that phytoene synthetase

is constitutive. It may only be partly constitutive because certainly phytoene synthesis is stimulated on photoinduction [300, 333–335]. By adding the inhibitor of protein synthesis, cycloheximide, at varying times after illumination, a differential inhibition of synthesis of phytoene,  $\zeta$ -carotene, neurosporene, lycopene, etc., is observed [309]. Furthermore, if mycelia after illumination are kept under nitrogen, which blocks carotenogenesis but not enzyme synthesis, and subsequently exposed to oxygen in the presence of cycloheximide, synthesis of all the carotenoids occurs as if cycloheximide had not been present [305]. These experiments suggest that light induces the simultaneous synthesis of the group of enzymes concerned with the formation of phytoene and its subsequent stepwise dehydrogenation, that is it possibly derepresses a carotenoid operon. This is supported to some extent by recent work on *N. crassa* [295]. An alternative possibility, that light induces phytoene synthetase and that the subsequent enzymes are induced by their substrate, appears to be ruled out.

It should be noted that in *Cephalosporium diospyros* the synthesis of a specific protein associated with light-induced carotenogenesis could not be demonstrated and inhibitors of protein synthesis had no effect on the induction [310].

Both hydrogen peroxide ( $10^{-1}$ – $10^{-2}$  M), *p*-hydroxy- and *p*-chloromercuribenzoate ( $5 \times 10^{-5}$  M) simulate photoinduction of carotenogenesis in that they induce synthesis in the dark [285, 287]. The  $H_2O_2$  and mercuribenzoate effects are sensitive to inhibitors of protein synthesis, but the mechanisms involved must be different from that concerned with photoinduction for the following reasons: (i) the effects of *p*-chloromercuribenzoate and of light are additive [311]; (ii) inhibition of the *p*-hydroxymercuribenzoate effect by thiols (e.g. cysteine, mercaptoethanol) does not affect photoinduction in the same cells [305]; (iii) in a mutant in which photoregulation has been repressed and which synthesizes carotenoids in the dark, addition of *p*-chloromercuribenzoate still stimulated additional pigment synthesis [287, 312, 313]. It appears that in *Fusarium* spp. a constitutive enzyme which normally shows only very slight activity is activated by *p*-chloromercuribenzoate whilst light induces a separate (isoenzyme) system [312]. The *p*-chloromercuribenzoate effect is observed in *Cephalosporium diospyros* [313] but not in *Verticillium agaricinum* [296], which also does not respond to  $H_2O_2$ . Antimycin A, which mimics the effect of light in some bacteria (Chapter 9) did not induce dark carotenogenesis in *V. agaricinum* although protein synthesis was stimulated [314]. The antibiotic was however stimulatory in *Leptosphaeria michotii* [186].

Azide inhibits photoinduced carotenogenesis in *F. aquaeductuum* as it does in *Mycobacterium* spp. (Chapter 9), probably by inhibiting a flavoprotein receptor [302]. An up-to-date review has recently been published [331].

## 8.3 FUNCTION IN REPRODUCTION

The function of carotenoids in photoresponses in fungi has been discussed in Chapter 3; here only function in reproduction will be considered.

It is some twenty-five years since detailed attention was drawn to the selective accumulation of carotenoid in reproductive organs of plants and animals. In the intervening years no clear function of carotenoids in reproduction has emerged in spite of considerable circumstantial evidence which is available. However, recent work on the Mucorales suggests that  $\beta$ -carotene is the precursor of the sex hormone trisporic acid (2.10). This compound was first discovered as a stimulator of  $\beta$ -carotene biosynthesis in *Blakeslea trispora*, *P. blakesleeana* and *Mucor mucedo* (Section 8.2.13(a)) and only later identified as the sex hormone extracted from *B. trispora* and *M. mucedo* [315–320] (see reviews by Gooday [321] and van den Ende [322] for full details). The conclusion that  $\beta$ -carotene can be converted into trisporic acid in *B. trispora* [252, 325–327] is supported by the observation of impaired sexual activity in carotene-deficient mutants of *P. blakesleeana* [321, 327]. The view that inhibition of both carotenogenesis and trisporic acid synthesis by diphenylamine [321] supports the idea that trisporic acid is synthesized from carotene cannot be sustained. Diphenylamine also inhibits sterol synthesis in *B. trispora* [250] and trisporic acid stimulates the synthesis of sterols and other polyenes [250, 321]. It is also to be noted that *Zygorhynchus molleri* synthesizes trisporic acid but not  $\beta$ -carotene [319] and is self-fertile. However, the labelling pattern derived experimentally in the C-1 group of zeaxanthin (3,3'-dihydroxy- $\beta$ -carotene) in a *Flavobacterium* is opposite to that deduced for trisporic acid in *B. trispora* (see Chapter 2).

The accumulation of  $\beta$ -carotene in the zygospores of *M. mucedo* [320] may be due to its proposed role as a precursor of sporopollenin, the resistant protective material in the outer wall of the zygospore. In *M. mucedo*,  $\beta$ -carotene is incorporated into sporopollenin [320] which appears to be a polymer of cross-linked xanthophylls. The ascospores of *Neurospora crassa* also contain sporopollenin;  $\beta$ -carotene is incorporated into it although the accumulation of pigment is not so great as in *M. mucedo*; furthermore carotenoid-deficient mutants, in which levels are considerably lower than in the wild type, also produce sporopollenin [323]. So we now have a possible rationale for the accumulation of carotenes in the gametangia of other fungi and it has been pointed out that  $\gamma$ -carotene synthesis in *Blastocladiella emersonii* is greater when the mould forms thick-coated resistant sporangia. On the other hand, as Burnett [324] indicates, carotene accumulation is not always related to either sporopollenin production or to sexual reproduction; for example,  $\gamma$ -carotene accumulates only in the male gametangium and gametes of *Allomyces* spp. [7] and only in male *Blastocladiella variabilis* [11].

It is claimed that carotenoids are involved in the photosensitive reaction which stabilizes the sporulation rhythm in *Leptosphaeria michotii* [325].

## 8.4 REFERENCES

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## NON-PHOTOSYNTHETIC BACTERIA

## 9.1 INTRODUCTION

Carotenoid distribution in non-photosynthetic bacteria resembles that in fungi in being sporadic and, at the moment, apparently unpredictable. Bacteria which are carotenogenic produce many pigments with specialized structures; these include the  $C_{30}$  carotenoids present in some Staphylococci and the  $C_{45}$  and  $C_{50}$  carotenoids found mainly in certain Flavobacteria. The appearance of carotenoid glycosides is also noticeable as is the relatively limited occurrence of  $\beta$ -carotene and related compounds, the absence of compounds with  $\epsilon$ -rings, except in the  $C_{45}$  and  $C_{50}$  carotenoids and perhaps in some Mycobacteria, and the almost complete absence of xanthophylls, including epoxides, characteristic of higher plants.

## 9.2 DISTRIBUTION

## 9.2.1 EUBACTERIALES

(a) *Micrococcaceae*

*Staphylococci*. Early work on various strains of *Staphylococcus aureus* indicated the presence of conventional  $C_{40}$  carotenoids (see First Edition of this book for early references) but the first intimation that these pigments were not what they seemed was the report that the phytoene present in *Staph. aureus* was not the usual  $C_{40}$  polyene but the  $C_{30}$  analogue termed 'bacterial phytoene' [1]. It is now clear from detailed studies that all the polyenes in *Staph. aureus* are  $C_{30}$  carotenoids provisionally termed diapocarotenoids [2, 3]. They include the  $C_{30}$  analogues of phytoene (1.36), phytofluene (1.37),  $\zeta$ -carotene (1.14), 7,8,11,12-tetrahydrolycopene and neurosporene (2.14), and termed diapophytoene (9.1), diapophytofluene (9.2), diapo- $\zeta$ -carotene (9.3), diapo-7,8,11,12-tetrahydrolycopene and diaponeurosporene (9.4), respectively. The 15,15'-*cis* isomer of (9.1) predominates. The main pigment is,

however, 4,4'-diaponeurosporen-4-oic acid (9.5) [4]; it is not rubixanthin (4.9) as previously reported [5-7]. 'Rubixanthin' emerges as an artefact, being the methyl ester of (9.5) formed during extraction of the pigment from the cells with alkaline methanol [8]. The pigments found in *Staph. aureus* 209P are listed in Table 9.1. *Staph. citreus*, on the other hand, contains sarcinene and sarcinaxanthin [8] [see under Micrococci] which are C<sub>50</sub> carotenoids. The diffuse variant of *Staph. aureus* contains less pigment than the compact variants [10] and strains both sensitive and resistant to chloramphenicol also vary in their carotenoid pattern [11].

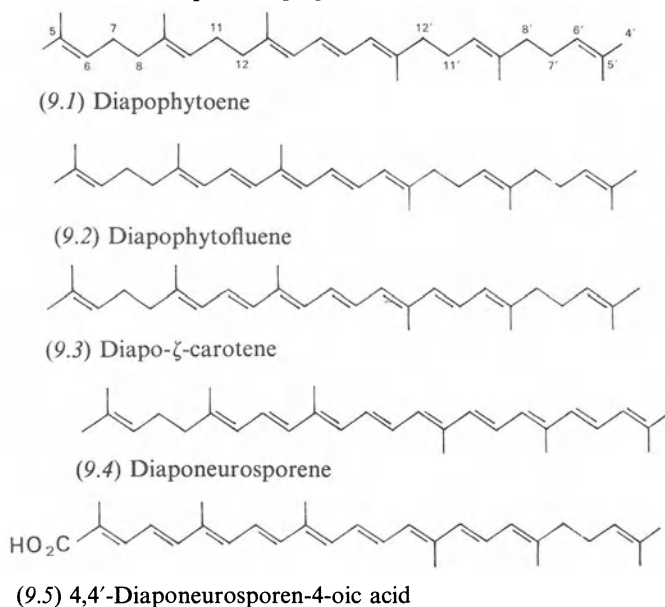


Table 9.1 Carotenoids of *Staphylococcus aureus* 209P[8]

Pigment	Relative amounts (% of total)
4,4'-Diapophytoene <sup>a</sup> (9.1)	74.79
4,4'-Diapophytofluene (9.2)	2.08
4,4'-Diapo-ζ-carotene (9.3)	1.84
4,4'-Diaponeurosporene (9.4)	0.67
4,4'-Diaponeurosporen-4-oic acid (9.5)	11.55 (1.81) <sup>b</sup>
4,4'-Diaponeurosporen-4-oic acid ester	1.69
4-Hydroxy-4,4'-diaponeurosporen-4-oic acid	0.59
4-D-Glucopyranosyloxy-4,4'-diaponeurosporen-4-oic acid	4.28
4-D-Glucopyranosyloxy-4,4'-diaponeurosporen-4-oic acid ester	0.76

Notes

<sup>a</sup>15-*cis* isomer

<sup>b</sup>*Cis*-isomer

*Sarcinae*. *Sarcina aurantiaca* produces lycopene (1.11) [12, 13] and  $\beta$ -carotene (1.13) [14, 15], one unknown xanthophyll and one reported to be zeaxanthin (3,3'-dihydroxy- $\beta$ -carotene) [13, 16, 17]. New techniques may well reveal the presence of  $C_{50}$  carotenoids rather than the conventional ones reported in the older literature. An extreme halophile *S. morrhuae* (?*Micrococcus morrhuae*) produces a water-soluble carotenoid glycoprotein [18, 18A] as well as the  $C_{50}$  bacterioruberin (1.78) and its derivatives, but no hydrocarbons or retinal (8.8) could be detected [19]. *S. littoralis*, on the other hand, produces hydrocarbons and retinal in addition to the bacterioruberins [19]. *S. flava* contains the same pigments as *S. lutea* [18A] [*S. lutea* has recently been redesignated *Micrococcus luteus* and is discussed in the next section].

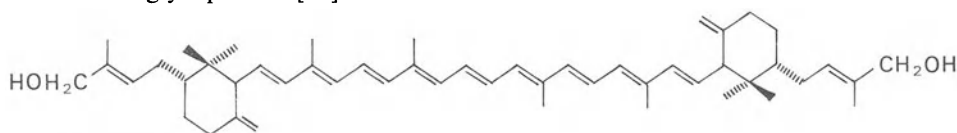
*Micrococci*. Unknown xanthophylls have been reported in *Micrococcus erythromyxa* [20], *M. flavus* [5], three marine *Micrococci* [21] and *M. lyso-deikticus* [22, 23]. Partial structures were reported for the four xanthophylls present in a radiation-resistant *Micrococcus* sp. [24] and keto-carotenoids are present in similar organisms, *M. radiophilus* and *M. radiodurans* [25]. The principal pigment in *M. roseus* is said to be canthaxanthin (1.40) [26–28] with echinenone (1.22) and 4-hydroxyechinenone [28, 29] as minor components. The carotenoids in a yellow halophilic coccus, H5B-2, consist of neurosporene (2.14) (81%), a lycopene-like pigment (4%) and a dihydroxy- $\zeta$ -carotene-like compound [30]. Another yellow coccus synthesized mainly the tertiary glycoside 1-hexosyl-1,2-dihydro-3,4-didehydro-apo-8'-lycopenoic acid in which the hexose is probably mannose [31]; this compound was originally reported as a methyl ester which was undoubtedly an artefact. A moderately halophilic coccus H<sub>5</sub>14033 (*Halococcus haloxanthus*) accumulates mainly phytoene (1.36) [19] whilst coloured carotenoids are present in the obligate halophile *Micrococcus morrhuae* [32]. The colour variants of *M. tetragenus* fall into five types: yellow, which contain lutein (1.74); mucoid-pink, which contain lycopene (1.11); pink, which contain rhodoxanthin (4.10) and pink-yellow and brown types both of which contain  $\gamma$ -carotene (1.38) and rubixanthin (4.9) [33]; the presence of lutein is unexpected and perhaps the identity of all the pigments reported should be checked by modern methods. Carotenoid-deficient mutants of *M. roseus* have recently been reported [28].

*Micrococcus luteus* was until recently identified as *Sarcina lutea*. From the carotenoid point of view it would appear to have closer connections with the *Sarcinae* than the *Micrococci*. It is characterized by the production of the  $C_{50}$ -diol sarcinaxanthin (9.6) [34], isomeric with decaprenoxanthin (Section 9.2.1.(f)); other pigments present include mono- and di-*D*-glucosides of sarcinaxanthin and compounds not fully characterized but considered to be 7,8-dihydrosarcinaxanthin and  $C_{45}$ -mono-ols [35, 36]. The nature of the hydrocarbon sarcinene reported in early investigations on *M. luteus* [13, 16, 17] is not known and in recent investigation it was not encountered [34]. A number of carotenoid mutants of *M. luteus* have been obtained and are listed

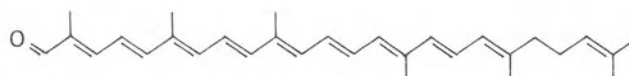
Table 9.2 Carotenoids in mutants obtained from *Micrococcus (Sarcina) luteus* ATCC 9341a [37, 38]

Number	Polyenes produced	Mutagen
2a	Normal in reduced amounts	ethyl-methyl-sulphonate
4b	Normal in reduced amounts	
7	Pigments with 8 conjugated double bonds	
93a	Colourless polyenes	u.v. light

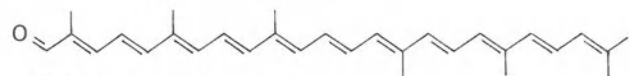
in Table 9.2. In a colourless mutant the properties of a number of enzymes located in the cell membrane are altered [39]. As in the case of *Sarcina morrhuae*, one of the *M. luteus* pigments appears to be in the form of a water-soluble glycoprotein [13].



(9.6) Sarcinaxanthin



(9.7) 4,4'-Diaponeurosporen-4-al



(9.8) 4,4'-Diapolycopen-4-al

### (b) *Neisseriaceae*

A series of C<sub>30</sub> carotenoids similar to those isolated from *Staphylococcus aureus* has been isolated from *Streptococcus faecium* UHN5641 (Table 9.3) [40, 41]. The main xanthophyll present, 4-hydroxy-4,4'-diaponeurosporene, occurs both free and as a 4-*D*-glucoside [41]. A *Streptococcus* sp. from the intestinal flora of sheep and chickens produced 29 µg carotenoid/g dry cells [42].

### (c) *Brevibacteriaceae*

*Brevibacterium* KY4313 growing on petroleum produces a number of carotenoids [43–45] the major component being canthaxanthin (1.40); β-carotene (1.13), echinenone (1.22), 15 *cis*-canthaxanthin, three carboxylic acids and three unidentified pigments are also present [44]. On the other hand, strain 103 produces mainly astaxanthin (1.68) [46]. An unidentified carotenoid-like pigment is also present in *Brevibact. linens* [47].

Table 9.3 Carotenoids in *Streptococcus faecium* UNH 564P [41]

Pigment	Relative amount (% of total pigments)
4,4'-Diapophytoene (9.1)	45.5
4,4'-Diapophytofluene (9.2)	13.3
4,4'-Diapo- $\zeta$ -carotene (9.3)	4.5
4,4'-Diapo-7,8,11,12-tetrahydrolycopene	1.1
4,4'-Diaponeurosporene (9.4)	13.8 (1.8) (1.1) <sup>a</sup>
4-Hydroxy-4,4'-diaponeurosporene	2.4
4-D-Glucopyranosyloxy-4,4'-diaponeurosporene	14.7
4,4'-Diaponeurosporen-4-al (9.7)	0.3
4,4'-Diapolycopene-4-al (9.8)	0.5

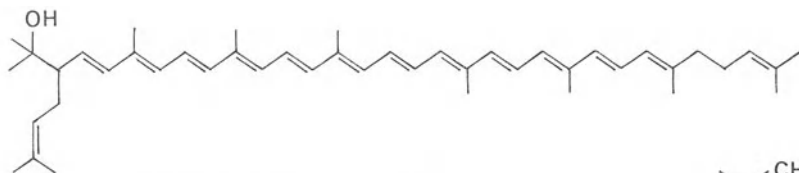
Note

<sup>a</sup>Cis-isomers

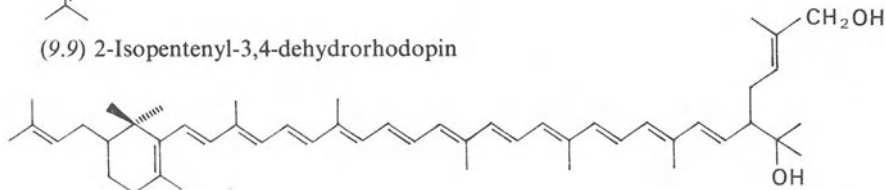
#### (d) *Corynebacteriaceae*

*Corynebacteria*. The pigments of *Corynebact. poinsettiae* include the C<sub>45</sub> compound 2-isopentenyl-3,4-dehydrorhodopin (9.9) [48] and the C<sub>50</sub> compounds bisanhydrobacterioruberin (2.18) [49], previously thought to be spirilloxanthin (see Chapter 10) [50]; C.p.450 (2.9) [49], previously described as  $\beta$ -cryptoxanthin (1.60) [50]; and C.p.473 (9.10) [49] earlier identified as lycopene (1.11) [50]. A strongly polar xanthophyll, corynexanthin, reported in 1954 in a marine *Corynebact.* sp. [51] is a monoglucoside of the C<sub>50</sub> carotenoid decaprenoxanthin (1.29) [52], first isolated from certain flavobacteria (see Section 9.2.1.(f)). One wonders whether the lycopene and cryptoxanthin noted in early work on *Corynebact. michiganense* were mis-identified [53].

The main pigment of a coryneform hydrogen bacterium [54], a *Corynebacterium* strain 7C [19] and *C. autotrophicum* [54A] is the dirhamnoside of zeaxanthin of which the absolute configuration has now been fully established [54A].



(9.9) 2-Isopentenyl-3,4-dehydrorhodopin



(9.10) C.p. 473



*Arthrobacteria*. An *Arthrobacter* sp. produces the mono- and diglycosides of decaprenoxanthin (1.29) [55]. *A. glacialis* produces decaprenoxanthin, bisanhydrobacterioruberin (2.18) and Ag.470 which probably combines one half of each of the two other pigments in its molecule [56]. Another *Arthrobacter* species synthesizes 4-keto- $\gamma$ -carotene [56A].

*Cellulomonads*. Carotenoid pigments in *Cellulomonas* spp. are characterized by decaprenoxanthin-like structures [57, 58].

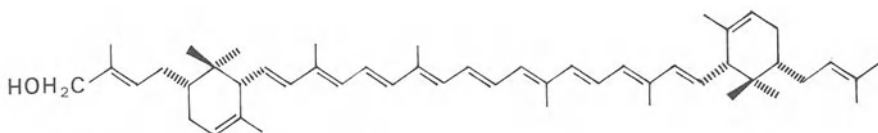
#### (e) *Rhizobiaceae*

The *Rhizobium* sp. which specifically nodulates *Lotononis bainesii* contains an unidentified carotenoid pigment with absorption maxima (hexane) at 470, 496 and 529 nm [59]. *Rhizobium lupini* has recently been examined in detail [59A], it synthesizes 2,3,2',3'-ditrans-tetrahydroxy- $\beta$ -carotene and 2,3,2',3'-ditrans-tetrahydroxy- $\beta$ -caroten-4-one as major pigments; smaller amounts of  $\zeta$ -carotene and  $\beta$ -carotene were detected. Caloxanthin and nostoxanthin (see Chapter 7) may also be present. The existence of carotenoids with one hydroxyl group at C-2 and one at C-3 is extremely rare; they have previously only been reported in the blue-green algae *Anacystis nidulans* (see Chapter 7).

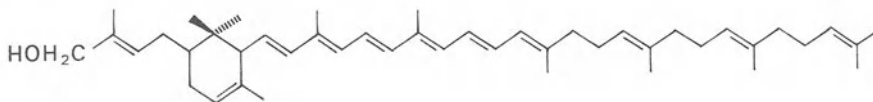
#### (f) *Achromobacteriaceae*

*Flavobacteria*. *Flavobacterium* (*Cellulomonas*) *dehydrogenans* was the first identified source of C<sub>50</sub> carotenoids and decaprenoxanthin (1.29) the first member of this group to be fully characterized [60–63]. Other pigments isolated from this organism include deshydroxydecaprenoxanthin (9.11) [58], the C<sub>45</sub> pigments nonaprenoxanthin (9.12) [58] and 11',12'-didehydrononaprenoxanthin [64]. The phytoene in *F. dehydrogenans* is the C<sub>40</sub> all *trans* species (1.36) [58, 60, 64A], the more usual isomer to accumulate in bacteria is the 15-*cis* compound.

In contrast to *F. dehydrogenans*, a *Flavobacterium* strain (alternatively named 0147 or R1519) accumulates a conventional C<sub>40</sub> pigment zeaxanthin [65–68] with the same absolute configuration at C-3 and C-3' as that from higher plants and algae [69]. Another strain (R1560) produces mainly zeaxanthin but in addition  $\beta$ -carotene (1.13),  $\gamma$ -carotene (1.38) neurosporene (2.14) [19],  $\beta$ -zeacarotene (2.13), lycopene (1.11), 3-hydroxy- $\beta$ -zeacarotene, rubixanthin (4.9) and  $\beta$ -cryptoxanthin (1.60) as well as the partly saturated polyenes phytoene (1.36) in its 15-*cis* form, phytofluene (1.37),  $\zeta$ -carotene (1.14) and 7,8,11,12-tetrahydrolycopene and also several apocarotenoids [70, 70A]. A *Flavobacterium* sp. from the intestinal flora of sheep and chickens contained 1.2–5.2  $\mu\text{g/g}$  dry cells [42].



(9.11) Deshydroxydecaprenoxanthin



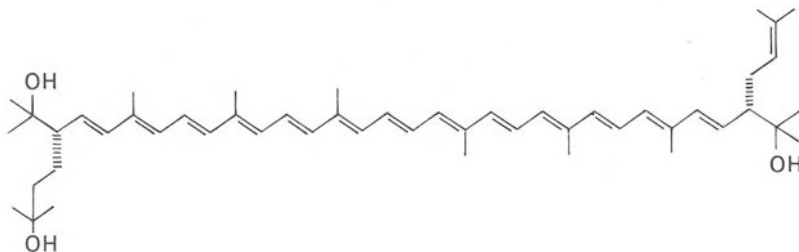
(9.12) Nonaprenoxanthin

## 9.2.2 PSEUDOMONADALES

### (a) *Pseudomonadaceae*

*Pseudomonads.* *Pseudomonas echinoides* synthesizes a xanthophyll considered to be 3,5,3'-trihydroxy-5,6-dihydro- $\beta$ -carotene. A series of mutants of this organism have been obtained [4]: (i) white, accumulating phytoene (1.36) and phytofluene (1.37); (ii) red, accumulating lycopene (1.11) and (iii) brown accumulating lycopene and  $\gamma$ -carotene (1.38) (probably) and the wild-type xanthophylls [71]. The so-called *Pseudomonas xanthe* produces decaprenoxanthin (1.29) [58]. Carotenoids have been detected in *Ps. phaseolicola* [72], and *Ps. extorquens* produces a pigment similar to rhodoxanthin (4.10) [73].

*Halobacteria.* There are a number of special features about carotenoids of the extreme halophiles, *Halobacterium cutirubrum* (various strains) [74, 75, 76], *H. halobium* [19] and *H. salinarum* [19, 49, 77]. They generally all produce the  $C_{40}$  hydrocarbons phytoene (1.36) and  $\beta$ -carotene (1.13) which are occasionally accompanied by lycopene (1.11), although the  $C_{30}$  diapo-phytoene (9.1) is present in one strain of *H. cutirubrum* [74]. On the other hand the xanthophylls present are  $C_{50}$  compounds, derivatives of the acyclic  $C_{50}$  bacterioruberin (1.78) (Table 9.4). Furthermore all these bacteria are characterized by the presence of a membrane retinal-protein complex named bacteriorhodopsin and first isolated from *H. halobium* [78, 79]. This corres-



(9.13) Anhydrobacterioruberin

Table 9.4 Carotenoids in some extreme halophiles (Halobacteria) [19]

Organism	Pigment <sup>a</sup>
<i>H. cutirubrum</i> 54001	1, 2, 3, 4, 5, 6, 7, 8
<i>H. halobium</i> 34020	1, 2, 4, 5, 6, 7
<i>H. halobium</i> M, 34014	1, 2, 3, 4, 5, 6, 7
<i>H. salinarum</i> 34002	1, 2, 4, 5, 6, 7
<i>H. salinarum</i> PN	1, 2, 3, 4, 5, 6

Key

1. Phytoene (1.36)	5. Monoanhydrobacterioruberin (9.13)
2. $\beta$ -Carotene (1.13)	6. Bisanhydrobacterioruberin (2.18)
3. Lycopene (1.11)	7. (?) Trianhydrobacterioruberin
4. Bacterioruberin (1.78)	8. Diapophytoene (9.2)

Note  
<sup>a</sup>All contain retinal

ponds to the visual pigment in animal eyes (see Vol. 2). A colourless mutant of *H. salinarum* PN produces only traces of the bacterioruberins [23]. A moderate halophilic bacterium from the Dead Sea contains bacterioruberin (20%) but the main pigment, C<sub>50</sub>H<sub>74</sub>O<sub>4</sub>, has not yet been fully characterized [80]. Another halophilic organism *Paracoccus haloxanthus* produces only phytoene (1.36) with traces of  $\beta$ -carotene (1.13) [19].

*Xanthomonads*. The pigments of *Xanthomonas juglandis* [81] and *Xanthomonas* sp. [82] which were originally thought to be carotenoids are not so but are bromine-containing polyenes [83].

9.2.3 ACTINOMYCETALES

(a) *Mycobacteriaceae*

Leprotene, a dehydro- $\beta$ -carotene, was first isolated from *Mycobact. leprae* in 1937 [84–86], but it was not until 1964 that it was identified with isorenieratene (9.14) [87], an aromatic carotene first obtained from the sponge *Reniera*. Leprotene is a constant component of almost all *Mycobacteria* examined, as is  $\beta$ -carotene (1.13). No conventional xanthophylls are observed; those which have been reported contain keto groups and hydroxyl groups with the hydroxyl groups glycosylated as in phleixanthophyll (9.16).

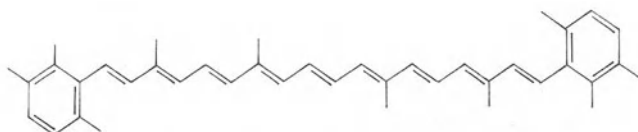
The carotenoid distribution in a number of *Mycobacteria* is given in Table 9.5. A number of carotenoid mutants of *Mycobact. phlei* have been reported [107], some of which have increased carotenoid levels [111]; this is particularly marked when N-nitrosomethylurea was the mutagen [112].

Table 9.5 Carotenoids in Mycobacteriaceae

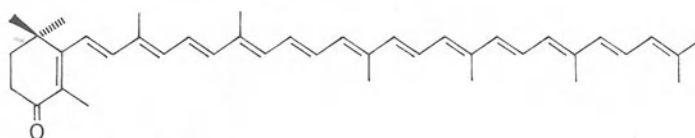
Organism	Pigments	Ref.
<i>Mycobact. aurum</i> (mutants)	1 + unidentified pigments	208
<i>Mycobact. bruynoghe</i> and <i>adant</i>	1	89
<i>Mycobact. carotenum</i>	5, 7	90
<i>Mycobact. citreochromogenum</i>	Present	91
<i>Mycobact. kansasii</i>	1, 2, 3, 4, 5, 6, 7, 8, 9	92, 93
<i>Mycobact. lacticola</i>	2, 4, 7, 8	94–98, 104
<i>Mycobact. leprae</i>	1	84–86
<i>Mycobact. luteum</i>	5, 7	90
<i>Mycobact. marinum</i>	5	98
<i>Mycobact. phlei</i>	1, 2, 3, 4, 5, 7, 8, 9, 10	15, 94, 99–108
<i>Mycobact. smegmatis</i> (? <i>lacticola</i> )	6, 11, 12	98, 109
<i>Mycobact. sp.</i>	acidic carotenoids	110

## Key

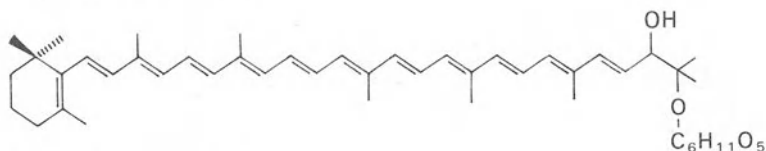
- |                               |        |                                |        |
|-------------------------------|--------|--------------------------------|--------|
| 1. Leprotene (isorenieratene) | (9.14) | 7. $\gamma$ -Carotene          | (1.38) |
| 2. Phytofluene                | (1.37) | 8. Lycopene                    | (1.11) |
| 3. $\alpha$ -Carotene         | (1.12) | 9. Phleixanthophyll            | (9.16) |
| 4. $\zeta$ -Carotene          | (1.14) | 10. 4-Ketophleixanthophyll     |        |
| 5. $\beta$ -Carotene          | (1.13) | 11. 4-Keto- $\gamma$ -carotene |        |
| 6. Neurosporene               | (2.14) | 12. 4-Ketotorulene             | (9.15) |



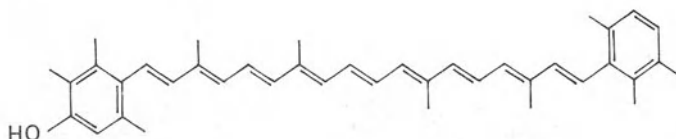
(9.14) Isorenieratene (Leprotene)



(9.15) 4-Ketotorulene



(9.16) Phleixanthophyll



(9.17) 3-Hydroxyisorenieratene

(b) *Streptomycobacteriaceae*

*Streptomyces mediolani* synthesizes not only isorenieratene (leprotene) (9.14) but also the first identified phenolic carotenoids 3-hydroxyisorenieratene and 3,3'-dihydroxyisorenieratene [113, 114]. Unidentified carotenoids have been reported in *S. antibioticus* [115, 116] and *S. erythraeus* [117]. A mutant *Streptomyces* related to *S. chrysomallus* makes  $\beta$ - and  $\gamma$ -carotenes and lycopene to the extent of 0.7 mg/g dry wt. [118].

(c) *Actinomycetaceae*

A *Nocardia* sp. contains a carotene-like pigment which is said to be not identical with either  $\alpha$ - or  $\beta$ -carotene or lycopene [119], although another strain synthesizes mainly  $\gamma$ -carotene (1.38) and 4-keto- $\gamma$ -carotene (G. Britton & T. C. Hutton, unpublished work). *Nocardia kirovani* synthesizes xanthophyll glycoside esters [120] whilst yet another strain of *Nocardia* produces  $\beta$ -carotene (1.13) [121]. A number of unnamed *Actinomycetes* have been reported also to contain unnamed carotenoids [122, 123] although one strain synthesized considerable amounts of lycopene (1.11) [124].

(d) *Myxococcaceae*

The characteristic pigments of *Myxococcus fulvus* are xanthophyll glycoside esters, in particular esters of myxobactin (1'-hydroxy-3',4'-didehydro-1',2'-dihydro- $\gamma$ -carotene glucoside) and of myxobactone (4-ketomyxobactin); other polyenes present are 4-ketotorulene (9.15), phytoene (1.36), phytofluene (1.37) and a number of unidentified pigments [125, 126]; myxobactone ester makes up 80% of the total pigments and represents 0.14% (dry wt.) of the bacterial membrane [127]. The pigment composition of *Chondromyces apiculatus* (Table 9.6) [128] is similar to that of *Sarangium compositum* [125] but does not contain the rhamnositides found in the latter; the absence of myxobactone is noteworthy because it is present in *Chondromyces* (*Stigmatella*) *aurantiaca* [125].

## 9.2.4 MICROPLASMATALES

Carotenoids are present in *Acholeplasma modicum* and *A. laidlawii* [129–133].

## 9.2.5 SPIROCHAETALES

(a) *Spirochaetaceae*

Two facultative anaerobic Spirochaetes produce carotenoids when cultured aerobically. *Spirochaeta aurantia* synthesizes mainly 1',2'-dihydro-1'-hydroxy-torulene which is also present in traces in Spirochaete RSI whose major

Table 9.6 Carotenoids in *Chondromyces apiculatus* [128]

Pigment	Percentage of total pigments present
$\gamma$ -Carotene (1.38)	38
Lycopene (1.11)	
1',2'-Dihydro- $\gamma$ -caroten-1'-ol	5
3',4'-Didehydro-1',2'-dihydro- $\gamma$ -caroten-1'-ol	
1',2'-Dihydro-1,2'-dihydrolycopene-1'-ol	
3',4'-Didehydro-1',2'-dihydro- $\gamma$ -caroten-1'-ol	2
3',4'-Didehydro-1',2'-dihydrolycopene-1',2'-diol acylate	
1'-Glucosyloxy-3',4'-didehydro-1',2'-dihydro- $\gamma$ -carotene ester	31
1'-Glucosyloxy-3',4'-didehydro-1',2'-dihydro- $\gamma$ -caroten-3-ol ester	19
1'-Glycosyloxy-3',4'-didehydro-1',2'-dihydrolycopene ester	5

pigment is 4-keto-1',2'-dihydro-1'-hydroxytorulene [134]. Similar pigments have only previously been reported in gliding bacteria (*Saprospira*) which have been considered under colourless algae (Chapter 7).

#### 9.2.6 MISCELLANEOUS

Table 9.7 lists bacteria which have been reported to contain unidentified carotenoids.

### 9.3 LOCALIZATION OF CAROTENOIDS

In *Micrococcus* (*Sarcina*) *luteus* [17, 149], *M. lysodeikticus* [17, 149], *M. morrhuae* [150], *M. roseus* [151], *Halobacterium halobium* and *H. salinarium* [152–154], and *Myxococcus fulvus* [155] carotenoids appear to be located in the cytoplasmic membrane and not the cell wall. Three types of membranes can be isolated from *H. salinarium*: (i) intracytoplasmic: (ii) purple coloured, containing retinal (8.8) in the form of bacterial rhodopsin [153, 156, 157] and (iii) orange coloured, containing the constituent carotenoids [53]. Carotenoids are also present in the membrane of *Acholeplasma laidlawii* [129–132] which does not have a cell wall. Sixty percent of the total outer membrane lipid of *Thermus aquaticus* consists of very polar carotenoids [158], although another investigation suggests that it is mainly  $\alpha$ -carotene (1.12) [159]. Furthermore a mutant strain with no carotenoids has no such outer membrane [160]. In a carotenoid-less mutant of *Staph. aureus* the activity of some components of the membrane (e.g. cytochrome oxidase) differs markedly from those in membranes from normal strains [161].

In *Micrococcus radiodurans*, however, the carotenoids are located in the

Table 9.7 Carotenogenic bacteria in which pigments have not been characterized

<i>Bacterium</i>	<i>Ref.</i>
<i>Bacterium mycoides</i>	14
<i>Bact. rubescens</i>	135, 136
<i>Corynebact. carotenii</i>	15, 137, 138
<i>Corynebact. fascians</i>	139
<i>Erwinia</i> spp.	15, 82
<i>Flavobact. arborescens</i>	14
<i>Flavobact. piscida</i>	140
<i>Flavobact. sp.</i>	82
Knallgas <i>Bacterium</i> 12/60/x	141
<i>Mycobact. flavum</i> var. <i>methanum</i>	142
<i>Mycobact. sp.</i>	143
<i>Mycobact. tuberculosis</i>	144
<i>Myxococcus flavus</i>	145
<i>Myxococcus virescens</i>	146
<i>Myxococcus xanthus</i>	147
<i>Pseudomonas methanica</i>	148
<i>Pseudomonas sp.</i>	147
<i>Streptococcus corallinus</i>	147

cell wall together with lipid, protein and polysaccharide in a fragile layer outside the mucopeptide layer and containing hexagonally packed subunits; a white mutant contained small amounts of this layer although no pigments [162]. A similar localization is reported for carotenoids in the 'Knallgas' *Bacterium*, 12/60/x [141].

## 9.4 FACTORS CONTROLLING SYNTHESIS

### 9.4.1 GENERAL

In *Mycobact. lacticola* (*smegmatis*) carotenoid synthesis at a temperature of 30°C parallels growth but continues after the latter has stopped [163]. However, in *Brevibacterium* KY 4313 it paralleled growth but did not continue after growth stopped [43].

### 9.4.2 CARBON SOURCES

Glycerol appears to be the most effective single carbon source for carotenogenesis in *Mycobact. phlei* and *Mycobact. rhodochrous* [164]. In *Mycobact. lacticola* (*smegmatis*) different carbon sources have a qualitative effect on carotenogenesis; on agar, for example, carotenes and neutral xanthophylls are formed whilst on mineral oil, carotenes and a highly polar acidic

carotenoid [possibly astaxanthin (*1.68*)], but no neutral xanthophylls, were produced [165]. Liquid paraffin is also a good medium for pigment synthesis by *Mycobact. carotenum* (carotenes and xanthophylls), *Mycobact. luteus* ( $\beta$ -carotene only) [166] and *Brevibacterium* KY 4313 [43]. A medium with a high carbohydrate content (4% mannitol) promotes carotenoid synthesis in *Corynebact. fascians* [167] and media with high C/N ratios increased the yield in *Brevibacterium* KY 4313 [43]. Furthermore, increasing glucose levels from 0.25–0.50% increases pigment production in *Strept. faecium* [168]. Pyruvate is strongly carotenogenic in *Micrococcus* (*Sarcina*) *luteus* but not in *Staph. aureus* [167], although acetate (0.2 M) is stimulatory in strain 209-P of the latter [169] and in *Acholeplasma laidlawii* [132]. Glucose stimulates carotenogenesis in another strain of *Staph. aureus* whilst malate and succinate are without effect [170]. A glucose concentration of 2–3% is optimum for pigment production in *Actinomyces antibioticus* [117] and two complex sources, corn extract and molasses, are excellent for carotenogenesis in *Mycobact. phlei*, and the  $\beta$ -carotene content can reach 50–60% of the total carotenoids present [171].

#### 9.4.3 NITROGEN SOURCES

In one strain of *Mycobact. phlei*, variation in nitrogen source did not affect carotenoid synthesis if the pH of the medium was controlled [172]. However, in another strain there was a marked drop in carotenoid formation when  $(\text{NH}_4)_2\text{SO}_4$  replaced asparagine, although the cultures were more pigmented [100]. In retrospect this would seem to be due to stimulated synthesis of carotenoid glycosides or peptides which would not have been extracted from the cell mass by the method in use twenty years ago. In another strain of *Mycobact. phlei* carotenogenesis is stimulated by the addition of peptides to the medium but not by asparagine or aspartic acid [173]. The best medium for carotenogenesis in *Actinomyces antibioticus* contains 0.75%  $(\text{NH}_4)_2\text{SO}_4$  [170], whilst  $\text{NH}_4\text{H}_2\text{PO}_4$  was a good source in *Brevibacterium* KY 4313 [43].

Leucine and valine can give rise to the carotenoid precursor HMG-CoA and thus in some cases, when the supply of HMG-CoA is limiting, stimulate carotenogenesis (see Chapter 2); this is not so in *Micrococcus* (*Sarcina*) *luteus* [166], *Corynebact. poinsettiae* [50], or *Brevibacterium* KY 4313 [43], but valine is stimulatory in *Corynebact. fascianus* [139]. Alanine and aspartic acid, but not glutamic acid, were stimulatory in *M. luteus* [166].

#### 9.4.4 MINERALS

Pigment production by *Mycobact. phlei* on a glucose-containing medium is stimulated by  $\text{Fe}^{3+}$  and inhibited by  $\text{K}^+$  and  $\text{PO}_4^{3-}$ ; however on a glycerol-based medium  $\text{K}^+$  is without effect, and  $\text{PO}_4^{3-}$  and  $\text{Fe}^{3+}$  inhibit synthesis [172]. In *Sarcina aurantiaca* [166]  $\text{Mn}^{2+}$  specifically stimulates caroteno-



genesis whilst  $\text{Fe}^{3+}$  has a non-specific effect on both growth and pigment production. Zn also stimulates pigmentation in this organism [173].

#### 9.4.5 LIGHT-PHOTOINDUCTION

Photoinduced carotenogenesis has been observed in a number of bacteria which are listed in Table 9.8. All these organisms synthesize only traces of carotenoid pigments in the dark, but after a short exposure to light considerable stimulation is observed under aerobic conditions. The action spectrum of this effect, which is also observed in some fungi (Chapter 8), suggests that the photoreceptor for a *Mycobact.* sp. is a flavin [174, 175]; for *Mycobact.*

Table 9.8 Bacteria in which photoinduced carotenogenesis occurs

Organism	Ref.
<i>Flavobacterium (Cellulomonas) dehydrogenans</i>	58
<i>Mycobact. flavum</i>	177
<i>Mycobact. kansasii</i>	92
<i>Mycobact. marinum</i>	171, 176, 181, 182
<i>Mycobact. sp.</i>	178, 183, 184
<i>Myxococcus xanthus</i>	174, 176, 179

*marinum* [175, 176] and *Myxococcus xanthus* [179] it is probably a porphyrin. The possibility remains that a carotenoprotein rather than a flavin is the receptor in *Mycobact. sp.* [see 185] although the observations that hydro-sulphite, which is known to reduce flavins, and azide, which complexes with transition metals which are required by many flavoenzymes, both inhibit photoinduction [174], support the view that a flavin is the active molecule.

The exact role of oxygen in photoinduction is still equivocal although most of the evidence points to direct participation in the photo-act [92, 186]. Antimycin A mimics the effects of light in *Mycobact. marinum* [187, 188] but *p*-chloromercuribenzoate and *p*-hydroxymmercuribenzoate, which have the same effect on photoinduction in fungi (see Chapter 8) have no effect in *Mycobact. marinum*. However, the exact loci of action of light and antimycin A must be different because of the cumulative effect of the two agents. Apparently the inhibitory action of antimycin A on electron transport is not involved in its photo-stimulatory effect [187, 188].

After exposure to light the events leading to carotenogenesis require a complete medium (C,N,P) and  $\text{O}_2$ , [92, 181, 183] although ferricyanide can replace oxygen in *Mycobact. sp.* for phytoene formation, which is pH and temperature-dependent [92]. There is a lag period between illumination and onset of carotenogenesis of 40–90 min in *Mycobact. sp.* [183], and of up to 4 h in *Mycobact. marinum* [188] but only of 8–10 min in *Mycobact. kansasii* [92].

Synthesis then continues for up to 14 h in *Mycobact. sp.* [183] and for up to 24 h in *Mycobact. marinum* [186]; in contrast antimycin A-stimulated synthesis in *Mycobact. marinum* continues for well over 48 h with a corresponding increase in the amount of pigment finally produced. A similar increase is observed in *Mycobact. marinum* and *Mycobact. sp.* exposed to continuous light or to saturating flashes at intervals of one hour or four hours, respectively [175, 186].

RNA synthesis increases during photoinduction [92] and thus protein synthesis is probably involved in the process, especially as the effect is inhibited by chloramphenicol and puromycin [174, 175, 178, 182, 188]. After photoinduction chloramphenicol has an inhibitory effect for only 4 h whilst after antimycin A-induction the inhibition lasts much longer. Thus it would seem that pigment induction is a result of derepression of genes controlling synthesis of enzymes involved in carotenogenesis. Antimycin A may act at a different site because, as just said, it tends to maintain continuous derepression whilst light maintains derepression only for about 4 h. Important detailed work [189, 190] has indicated that the enzyme most intimately concerned in photoinduction is prephytoene pyrophosphate synthetase (Chapter 2) the synthesis of which is wholly induced by photoinduction in *Mycobact. sp.* Geranylgeranyl pyrophosphate synthetase is also increased several fold but is present in non-induced cultures. Isopentenyl pyrophosphate isomerase is unaffected.

Although carotenoids are associated with cell membranes of photo-inducible bacteria, photoinduction does not result in additional synthesis of cell membranes [191].

Considerable stimulation of carotenoid synthesis in the presence of light has been observed in *Mycobact. lacticolum* [192] but it is not known whether photoinduction is involved. Qualitative differences have been observed in *Mycobact. phlei* strains PN and PNR which produce carotenes in the dark; on illumination of the cultures the carotenes are converted into xanthophylls [193, 194].

#### 9.4.6 TEMPERATURE

Relatively low incubation temperatures favour carotenogenesis in *Sarcina aurantiaca* [195], *Mycobact. phlei* [99, 172] and certain marine coryneforms [51]. In *S. flava* the optimum temperature is 34°C for growth and 25°C for carotenogenesis [21, 22]. *Micrococcus radiodurans* grows well between 25°C and 40°C, with 25–30°C the optimum range for carotenogenesis [196].

#### 9.4.7 pH

No marked effect on carotenoid formation was noted over the narrow pH range which allows growth of *Sarcina aurantiaca* [195], *Mycobact. phlei* [100] and *Brevibacterium* KY 4313 [43].

## 9.4.8 GROWTH FACTORS

Thiamine appears to play a significant part in carotenogenesis in a number of bacteria, but the exact site of action is unknown. Pigmentation of *Bacillus lombardo-pelligrini* and *B. boquet* is directly proportional to the thiamine content of the medium [197]. On a medium low in thiamine (0.1  $\mu\text{g}/100\text{ ml}$ ) *Corynebact. poinsettiae* accumulates mainly acyclic carotenoids in particular bisanhydrobacterioruberin (2.18); on a medium high in thiamine, bisanhydrobacterioruberin tends to disappear and to be replaced by the cyclic C.p. 450 (2.19) not found in low-thiamine cultures; *Corynebact. michiganense* behaves rather similarly [198]. In both *Mycobact. luteum* and *Nocardia corallina* maximum carotenogenesis was observed in a medium containing 1–5 and 0.1–1.0  $\mu\text{g}/\text{ml}$  thiamine, respectively. Hydrocarbon carotenoids predominated in the cells and it was the levels of these rather than of the minor xanthophylls which dropped when thiamine levels were reduced [199]. Vitamin B<sub>12</sub> was the only one of a number of water-soluble vitamins tested which stimulated carotenogenesis in *Brevibacterium* KY 4313 [43].

## 9.4.9 INHIBITORS

Inhibition of carotenogenesis by the well known inhibitor diphenylamine has been described in a number of bacteria (Table 9.9). Inhibition is accompanied by an accumulation of phytoene (1.36) (Chapter 2). The effects of a number of other compounds, including antibiotics, on carotenoid formation are given in Table 9.10.

Table 9.9 Bacteria in which carotenogenesis is inhibited by diphenylamine

Organism	Ref.
<i>Acholeplasma laidlawii</i>	132
<i>Actinomyces subflavus</i>	200
<i>Flavobacterium (Cellulomonas) dehydrogenans</i> <sup>a</sup>	58
<i>Mycobacterium phlei</i>	201, 202
<i>Myxococcus fulvus</i>	203
<i>Pseudomonas extorquens</i>	204
<i>Sarcina (Micrococcus) lutea</i>	205
<i>Staphylococcus aureus</i>	206
<i>Streptomyces antibioticus</i>	115
<i>Streptomyces erythraeus</i>	117

*Note*

<sup>a</sup>No inhibition with a *Flavobacterium* which synthesizes zeaxanthin [67].

Table 9.10 Effect of various compounds, including antibiotics, on carotenogenesis in bacteria

Compound	Organism	Effect	Ref.
Chloramphenicol	<i>Sarcina lutea</i> <sup>c</sup>	Inhibits, particularly xanthophylls	205
CTPA <sup>a</sup>	<i>Myxococcus fulvus</i>	Inhibits cyclization reactions	209
Iodoacetamide	<i>Streptomyces erythraeus</i>	Stimulates	117
Nicotine	<i>Mycobacterium</i> spp.	Inhibits cyclization reactions	210
	<i>Flavobacterium</i> spp.		67
	<i>Micrococcus</i> spp.		211
	<i>Amoebobacter morrhuae</i>	Inhibits conversion of lycopene into	212
	<i>Halobacterium cultirubrum</i>	C <sub>45</sub> and C <sub>50</sub> carotenoids	213
	<i>Halobacterium halobrum</i>		212
	<i>Sarcina litoralis</i>		212
Oxytetracycline	<i>Sarcina lutea</i> <sup>c</sup>	Inhibits, particularly xanthophylls	205
Penicillin	<i>Sarcina lutea</i> <sup>c</sup>	Inhibits, particularly xanthophylls	214
Penicillin G	<i>Brevibacterium</i> KY4313	Inhibits when added during later growth phases	43
Polymixin B	<i>Sarcina lutea</i> <sup>c</sup>	Inhibits, particularly xanthophylls	205
San 6706 <sup>b</sup>	<i>Myxococcus fulvus</i>	Inhibits with accumulation of phytoene	203, 209, 214
Streptomycin	<i>Sarcina lutea</i> <sup>c</sup>	Inhibits, particularly xanthophylls	205
Thallium acetate	<i>Acholeplasma laidlawii</i>	Inhibits	132

## Notes

<sup>a</sup> 2-(4-Chlorophenylthio) triethylamine hydrochloride.<sup>b</sup> 4-Chloro-5-(dimethylamino)-2- $\alpha,\alpha,\alpha$  (trifluoro-*m*-toluyl)-3-(2H)-pyridazinone.<sup>c</sup> Now *Micrococcus*.9.5 BIOSYNTHESIS OF C<sub>30</sub> CAROTENOIDS

The C<sub>30</sub> carotenoids of *Strept. faecium* and *Staph. aureus* are mevalonate-derived and appear to be formed as such and not as degradation products of C<sub>40</sub> carotenoids. Plausible biogenetic schemes, which involve dehydro-squalene, the C<sub>30</sub> analogue of phytoene, the first C<sub>40</sub> hydrocarbon in the formation of C<sub>40</sub> carotenoids, have been suggested for the formation of C<sub>30</sub> carotenes and xanthophylls (free, esterified and glycosylated) (Figs. 9.1 and

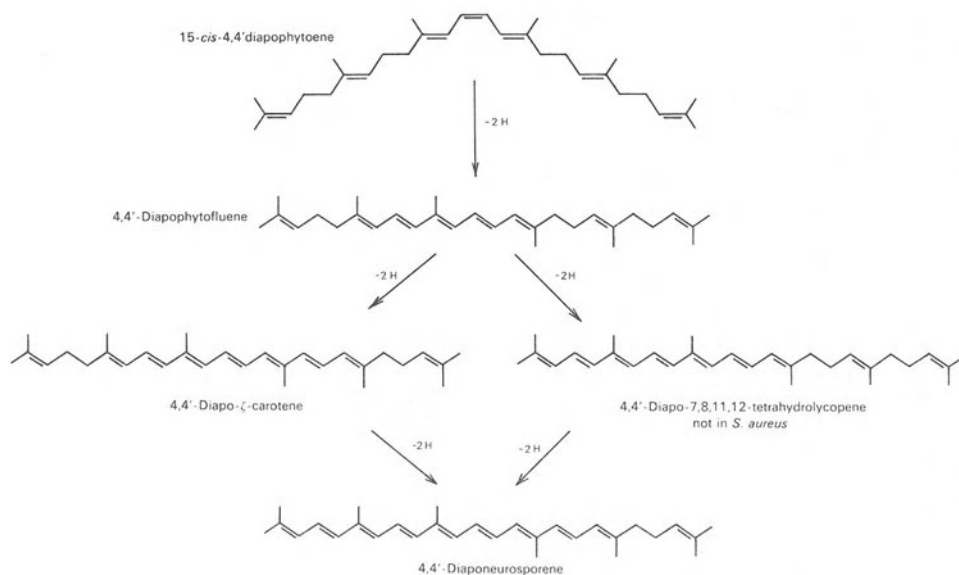


Fig. 9.1. Probable pathway of synthesis of  $C_{30}$  carotenoids in *Staphylococcus aureus* and *S. faecium* [8].

9.2) [8]. Little direct experimental evidence is yet available to substantiate the individual steps in these pathways, although it should be noted that *cis*-4,4'-diapophytoene rather than the *trans*- compound appears to be the first  $C_{30}$  precursor.

## 9.6 BIOSYNTHESIS OF $C_{45}$ AND $C_{50}$ CAROTENOIDS

When *Flavobacterium dehydrogenans* is cultured in the presence of diphenylamine the  $C_{40}$  polyenes phytoene (1.36), phytofluene (1.37),  $\zeta$ -carotene (1.14) and 7,8,11,12-tetrahydrolycopene (an unsymmetrical isomer of  $\zeta$ -carotene) accumulate at the expense of the  $C_{50}$  carotenoid decaprenoxanthin (1.29). If the inhibitor is washed out and the cells are resuspended in fresh medium then decaprenoxanthin is synthesized and the more unsaturated polyenes disappear. This suggests that  $C_{50}$  pigments are formed conventionally from  $C_{40}$  precursors and possible pathways to decaprenoxanthin are indicated in Fig. 9.3 [58]. A  $C_5$  unit could be added with cyclization at one end of the molecule to yield from 7,8,11,12-tetrahydrolycopene, neurosporene and lycopene  $C_{45}$  hydrocarbons corresponding to nonaprenoxanthin, 11',12'-dehydrononaprenoxanthin and P452, respectively. After hydroxylation nonaprenoxanthin and 11',12'-didehydrononaprenoxanthin are dehydrogenated to form P452. The addition reaction can then proceed at the other end of the molecule to yield eventually decaprenoxanthin. Decaprenoxanthin (1.29) contains  $\epsilon$ -rings, but other  $C_{50}$  carotenoids have  $\beta$ -rings (e.g. C.p. 450)

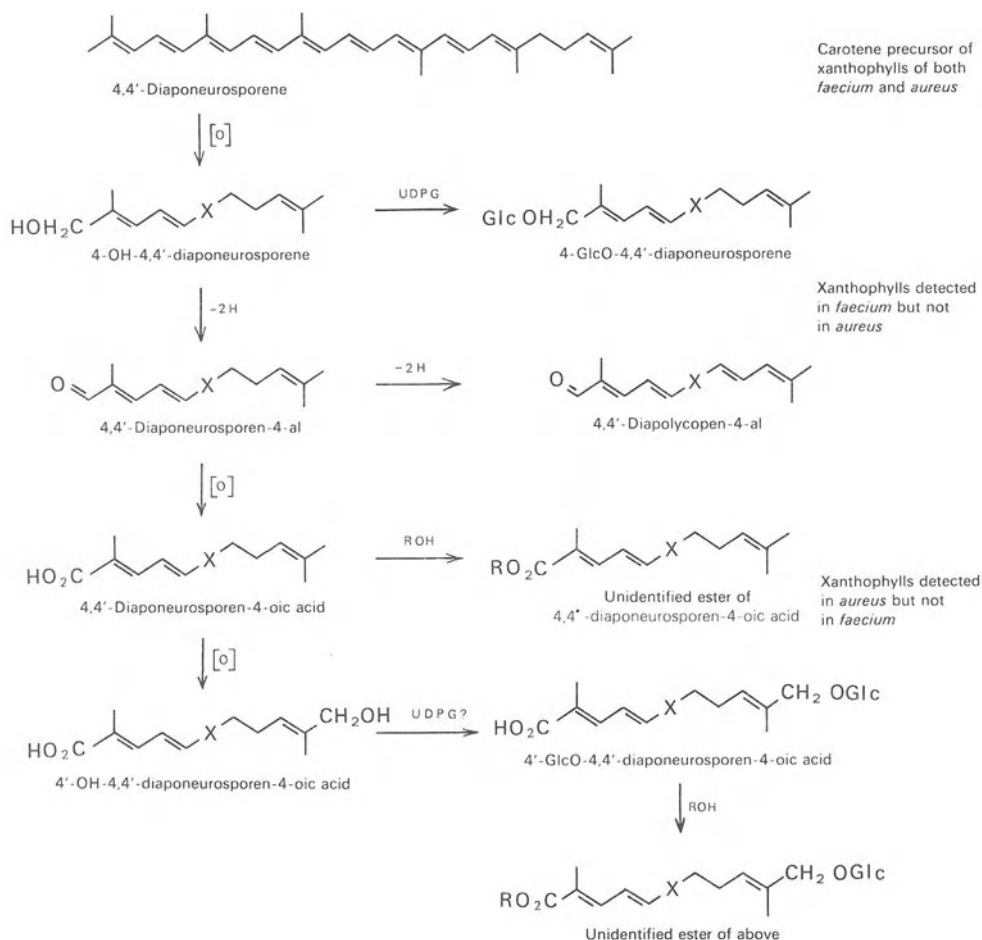


Fig. 9.2. Possible pathways for the biosynthesis of C<sub>30</sub> xanthophylls in *Staphylococcus aureus* and *S. faecium* [8].

(2.9) and  $\gamma$ -rings [e.g. sarcinaxanthin, (9.6)]. The mechanism for the production of these rings is a simple extension of that demonstrated for the synthesis of such rings in the C<sub>40</sub> series [215] (Fig. 9.4) (see Chapter 2); the only difference is that isopentenyl pyrophosphate takes the place of a proton as the electrophilic attacking species. The only experimental evidence for any of the steps outlined in Fig. 9.4 is a kinetic study which indicates that P452 is a precursor of decaprenoxanthin [216].

The existence of acyclic C<sub>50</sub> carotenoids means that additional C<sub>5</sub> units can be added to a C<sub>40</sub> precursor without cyclization taking place. It would seem that in *Halobacterium* spp., 3,4,3',4'-tetrahydrolycopene is the precursor of bacterioruberin (1.78) and that the insertion of the double bonds

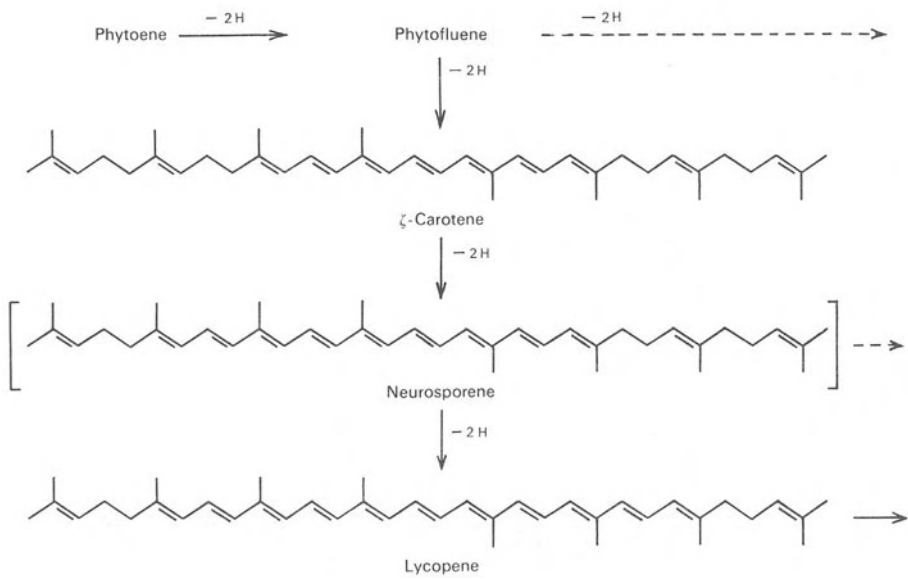
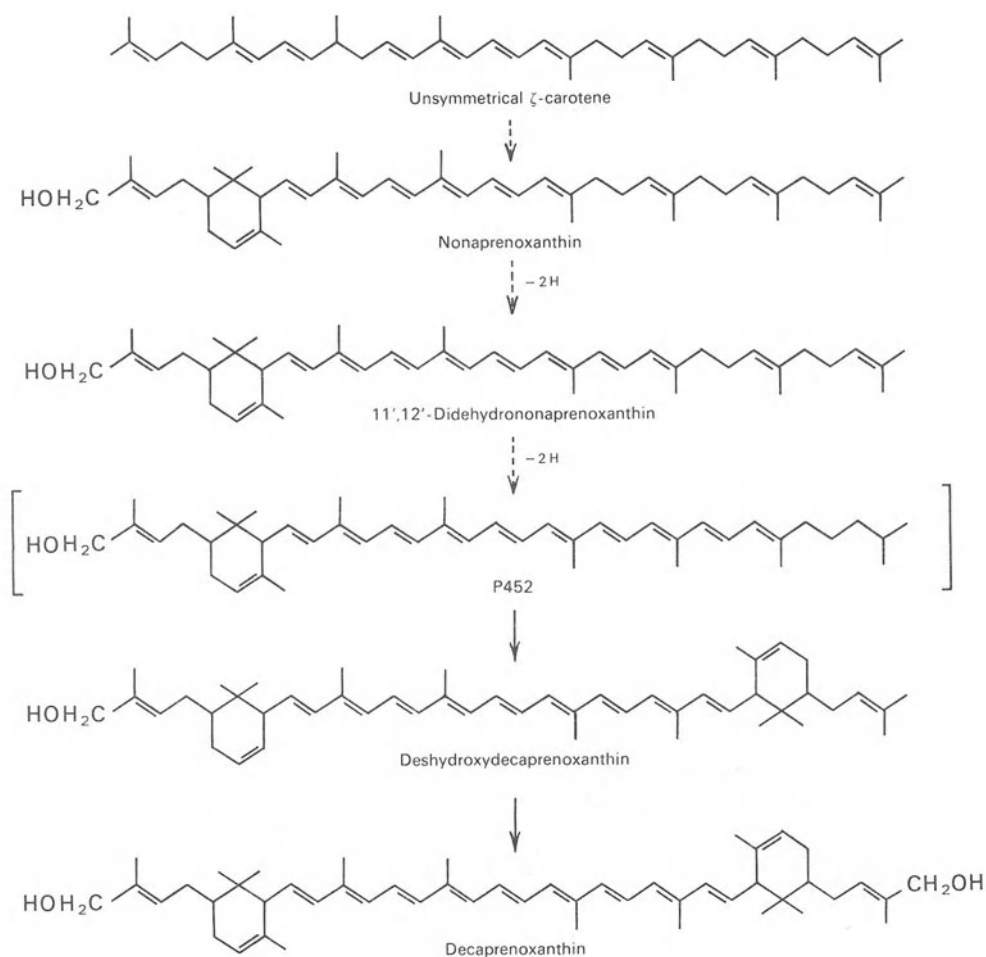


Fig. 9.3. Probable pathway for synthesis of  $C_{45}$  and  $C_{50}$  carotenoids.





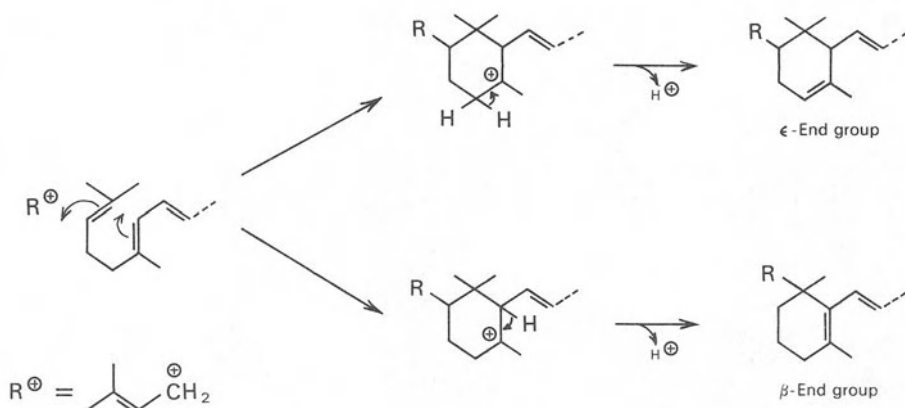


Fig. 9.4. Mechanism for synthesis of C<sub>45</sub> and C<sub>50</sub> carotenoids with β- and ε-rings from C<sub>40</sub> acyclic precursors.

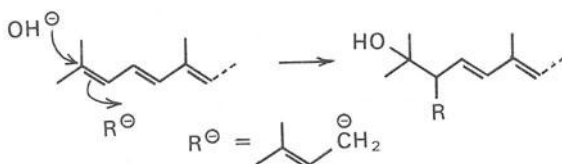


Fig. 9.5. Mechanism for synthesis of acyclic C<sub>45</sub> and C<sub>50</sub> carotenoids from C<sub>40</sub> acyclic precursors.

at 3 and 3' effectively prevents cyclization (Fig. 9. 5). Thus the existence of such acyclic pigments is presumably due to the presence in *Halobacterium* spp. of a 3,4-dehydrogenase which is not present in *F. dehydrogenans*. It is, however, an enzyme which is well distributed amongst the photosynthetic bacteria (Chapter 10).

## 9.7 FORMATION OF CAROTENOID GLUCOSIDE ESTERS

Experiments with inhibitors indicate that the pathway of synthesis of the unusual carotenoid glucoside esters in *Myxococcus fulvus* is as indicated in Fig. 9.6 [214]. The proposed branch point, neurosporene, accounts for the constant ratio of 4-ketotorulene to myxobactone ester observed under all experimental conditions. The sites of action of various inhibitors are indicated and the final step appears to be the insertion of oxygen to form the 4-ketoderivatives.

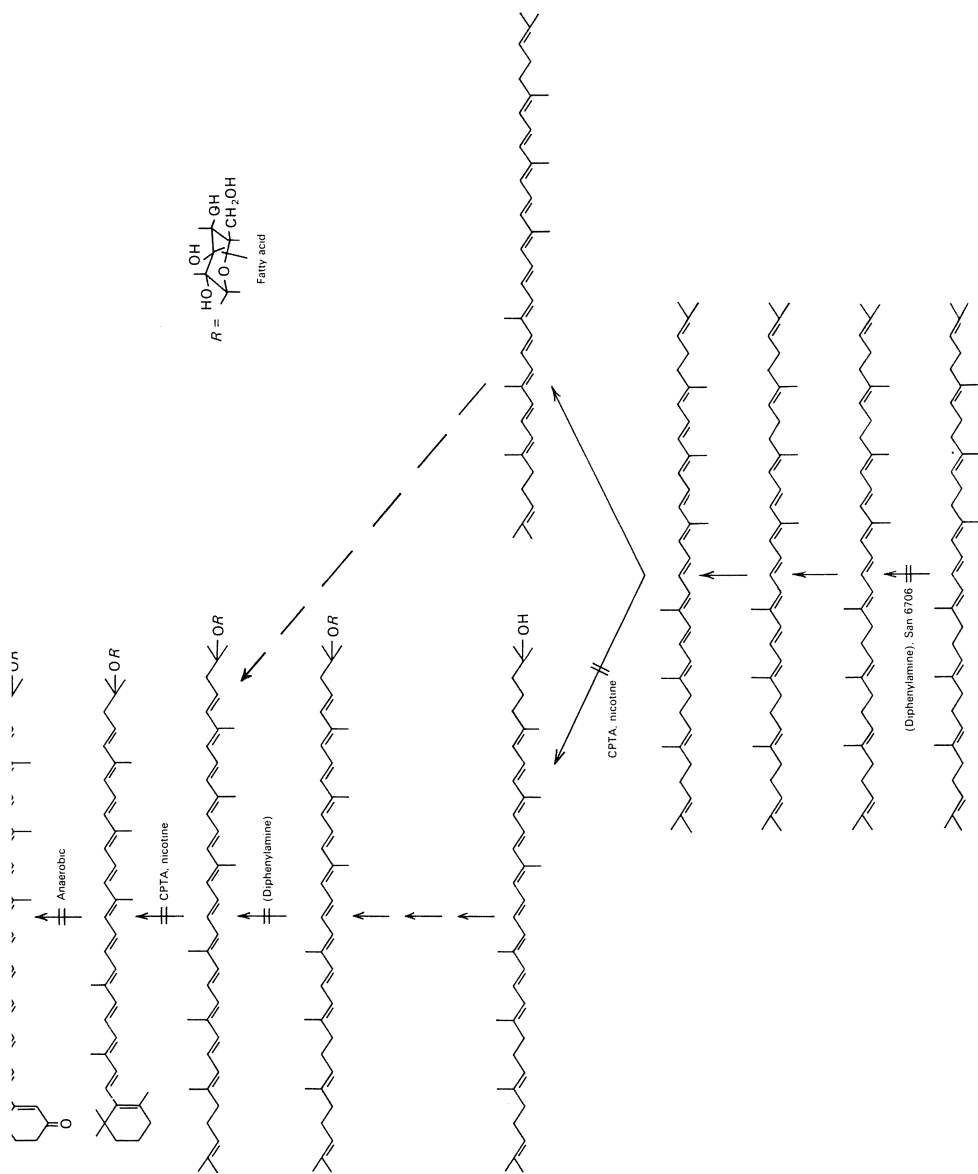


Fig. 9.6. The probable pathway of myxobactone in *Myxococcus fulvus*. The sites of action of various inhibitors are also indicated [214].

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## PHOTOSYNTHETIC BACTERIA

## 10.1 DISTRIBUTION

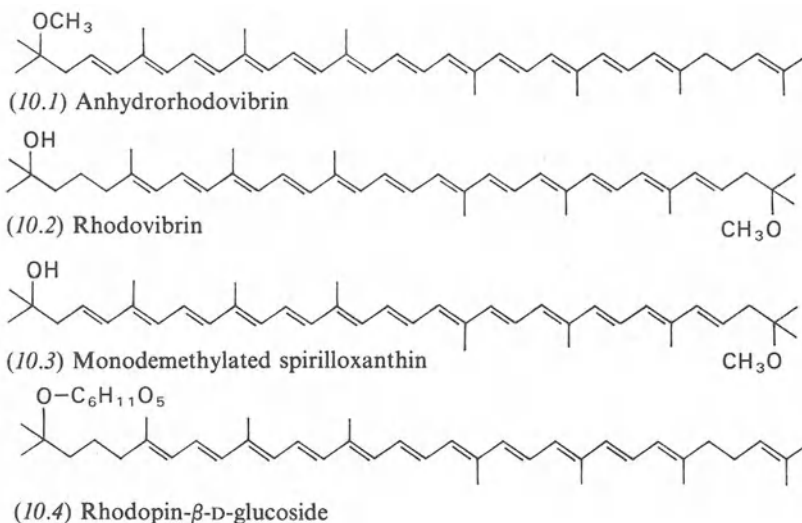
## 10.1.1 PURPLE PHOTOSYNTHETIC BACTERIA

The purple photosynthetic bacteria belong to two families, the Rhodospirillaceae [1] (previously Athiorhodaceae) and the Chromatiaceae [1] (previously Thiorhodaceae) according to whether or not they require a reduced form of sulphur for growth. Although they differ in detail their carotenoids have certain characteristics such as: (i) they are, with a few exceptions, acyclic; (ii) they have tertiary hydroxyl groups at C-1 and/or C-1', which are frequently methylated; (iii) they contain double bonds at C-3,4 and/or C-3',4' and (iv) they can contain keto groups at C-2 and/or C-2' and at C-4.

(a) *Rhodospirillaceae*

The carotenoid distribution in the Rhodospirillaceae is given in Table 10.1. These organisms have been roughly divided into three groups according to their pigment distribution [2, 3, 4]. Group 1 synthesizes the 'normal' spirilloxanthin (1.19) series: Group 2 synthesizes the 'alternative' spirilloxanthin series (see Section 10.4.1) and, under aerobic conditions, conjugated ketones such as spheroidenone (1.42); Group 3 cannot carry through all the steps involved in the conversion of lycopene into spirilloxanthin (see Figs. 10.4 and 10.6B) and their end products are such pigments as rhodopin (2.16). However, as might be expected, some deviations from these simple patterns are continually coming to light. Variations which should be emphasized are: (i) the occurrence in *Rhodopseudomonas acidophila* of carotenoid glucosides [34]; (ii) the presence of cross-conjugated carotenoids such as rhodopin-20-al (2.21) in, for example *Rps. acidophila* although they occur only in the strictly anaerobic strains of this organism; (iii) the presence in *Rps. viridis* of a series of hydrocarbons in which the terminal double bond (C-1,2) has undergone

saturation rather than hydration [20, 21] as occurs in the carotenoids of all other Rhodospirillaceae so far examined: (iv) the formation in *Rhodomicrobium vannielii* of  $\beta$ -carotene (1.13) and  $\beta$ -cryptoxanthin (1.60) [9] and of  $\beta$ -carotene in *Rps. acidophila* [5, 11], the only bicyclic carotenoids known in the Rhodospirillaceae: and (v) a pigment, 1-methoxy-3',4'-didehydro-1',2'-dihydro- $\beta$ , $\psi$ -carotene, which has one terminus uncharacteristically cyclic and the other with the 1-methoxy-1,2-dihydro structure characteristic of the Rhodospirillaceae, is also present in *Rm. vannielii* [19].



When the Rhodospirillaceae are cultured in the presence of the inhibitor diphenylamine, which inhibits desaturation of phytoene, then novel pigments can be detected which are not present in significant amounts in normal cultures. It is difficult to decide whether these compounds are normal metabolites which are very active biosynthetic intermediates or are produced as side reactions which only occur in the presence of the inhibitor. This problem will be discussed later in the chapter but the pigments detected only in DPA-inhibited cultures are listed in Table 10.2. The phytoene in *Rhodospirillum rubrum* appears to be almost entirely the *trans* isomer [33] whereas in *Rm. vannielii* about 30% of the *cis* isomer is also present [35].

**Mutants.** Some twenty years ago a mutant of *Rps. sphaeroides* was obtained which accumulated phytoene (1.36) only [38]. Since then other mutants of the same organism have been reported which accumulate mainly neurosporene (2.14) or neurosporene plus chloroxanthin (1-hydroxy-1,2-dihydro-neurosporene) [39–42]: in the first case insertion of a hydroxyl group (probably hydration), methylation and insertion of a keto group at C-4 are blocked, whilst in the latter hydration of neurosporene but no further steps can occur.

Table 10.1 Distribution of carotenoids in the Rhodospirillaceae

Organism	Group	Pigments	Ref.
<i>Rhodocyclus purpureus</i>	3	1, 2, 3, 4, 5	5
<i>Rhodomicrobium vannielii</i>	1	1, 2, 6, 7, 8, 9, 10, 11, 12, 13, 14	6–10
<i>Rhodopseudomonas acidophila</i> (8 strains)	1	5, 6, 8, 11, 15, 16, 17, 18, 19, 20	Present in some strains Present in all strains
<i>Rps. capsulata</i>	2	1, 2, 3, 4 1, 2, 10, 15, 21, 22, 23, 24	23 and 24 only produced in presence of O <sub>2</sub>
<i>Rps. gelatinosa</i>	2	1, 6, 7, 15, 21, 22, 23, 24, 25, 26, 27, 28	12, 13
<i>Rps. globiformis</i>	3	14, 15, 29, 30, 31, 32, 33	3, 12, 14, 15
<i>Rps. palustris</i>	3	1, 2, 6, 7, 8, 9, 17, 18, 25, 26, 34	17, 18 present only in young cultures
<i>Rps. sphaeroides</i>	2	?1, 15, 21, 22, 23, 24, 25, 27, 35, 36, 44, 45	23 and 24 only produced in presence of oxygen; 35 produced only in presence of nicotine
<i>Rps. viridis</i>	3	1, 5, 15, 18, 35, 36, 37, 38, 39, 40, 41, 42, 43, 46	10, 14, 15, 17–19, 18A
<i>Rhodospirillum fulvum</i>	3	1, 2, 9	20–25
<i>Rsp. molischianum</i>	3	1, 2, 15	11, 26
<i>Rsp. photometricum</i>	3	1, 2, 6, 7, 99	12, 26
<i>Rsp. rubrum</i>	1	6, 7, 8, 9, 10, 15, 16, 17, 18, 22, 23, 25	12
<i>Rsp. tenue</i> (4 strains)	3	1, 2, 3, 4	16, 17, 18 present only in young culture
			1, 2, 23, 25–33
			5

# Key

1. Lycopene (1.11)
2. Rhodopin (2.16)
3. Rhodopin-20-ol (= Warmingol) (2.20)
4. Rhodopin-20-al (= Warmingone) (2.21)
5. 13-*Cis*-lycopen-20-al (= Anhydrowarmingone) (10.1)
6. Anhydrotirodovibrin (= P481) (10.2)
7. Rhodovibrin (= Hydroxy P481) (10.3)
8. Monodemethylated spirilloxanthin
9. 1,2,1',2'-Tetrahydro-1,1'-dihydroxylycopene (1.19)
10. Spirilloxanthin (= Rhodoviolascin) (1.13)
11.  $\beta$ -Carotene (1.60)
12. 3,4-Didehydrorhodopin
13.  $\beta$ -Cryptoxanthin (2.14)
14. 1'-Methoxy-3',4'-didehydro-1',2'-dihydro- $\beta$ ,  $\psi$  carotene (1.36)
15. Neurosporene (1.37)
16. Phytoene (1.14)
17. Phytofluene (10.4)
18.  $\zeta$ -Carotene (1.41)
19. Rhodopin  $\beta$ -D-glucoside (1.42)
20. Rhodopin-20-al  $\beta$ -D-glucoside
21. Spheroideene
22. Hydroxyspheroideene (1',2'-dihydro-1'-hydroxyspheroideene)
23. Spheroideone (1.42)
24. Hydroxyspheroideone (1',2'-dihydro-1'-hydroxyspheroideone)
25. Chloroxanthin (1-hydroxy-1,2-dihydrodroneurosporene)
26. 2-Ketorhodovibrin
27. 2,2'-Diketospirilloxanthin (= P512, = P518)
28. Hydroxy P518
29. 7,8,11,12-Tetrahydrolycopene (unsymmetrical  $\zeta$ -carotene)
30. 1-Methoxy-1,2-dihydrolycopen-4-one
31. 1,1'-Dimethoxy-1,2,1',2'-tetrahydrolycopen-4-one
32. 1,1'-Dimethoxy-1,2,1',2'-tetrahydrolycopen-4,4'-dione
33. 1-Methoxy-1'-hydroxy-1,2,1',2'-tetrahydrolycopen-4-one
34. 3,4-Didehydrorhodopin
35. 1,2-Dihydro- $\zeta$ -carotene
36. 1,2,7,8,11,12-Hexahydrolycopene
37. 1,2-Dihydrodroneurosporene
38. 1,2-Dihydrolycopene
39. 1,2-Dihydro-3,4-didehydrolycopene
40. 1,2-Dihydrophytoene
41. 1,2-Dihydrophytofluene
42. 1,2,1',2'-Tetrahydrodroneurosporene
43. 1,2,1',2'-Tetrahydrolycopene
44. Methoxyspheroideene
45. Methoxyspheroideone
46. 3',4'-Didehydro-1,2-dihydrolycopen-20(or 20')-al

Table 10.2 Pigments noted in diphenylamine-inhibited cultures of the Rhodospirillaceae

Organism	Pigment	Ref.
<i>Rhodomicrobium vannielii</i>	1, 2, 3, 4, 5, 6, 7, 8	9
<i>Rhodopseudomonas globosa</i>	9, 10, 11	36
<i>Rps. palustris</i>	2, 3, 12, 13	12, 16
<i>Rps. viridis</i>	2, 13, 14, 15, 16, 17	21
<i>Rhodospirillum rubrum</i>	4, 5, 6, 7, 18, 19, 20, 21, 22, 23, 24	33, 35, 37

## Key

- |   |  |
|---|--|
| 1. Neurosporene (2.14)  | 12. Lycopene (1.11)  |
| 2. Phytoene (1.36)  | 13. $\zeta$ -Carotene (1.14)                                 |
| 3. Phytofluene (1.37)   | 14. 1,2-Dihydrophytoene                                      |
| 4. 7,8,11,12-Tetrahydrolycopene                                 | 15. 1',2'-Dihydrophytofluene                                 |
| 5. 1-Hydroxy-1,2-dihydrophytoene                                | 16. 1,2,1',2'-Tetrahydroneurosporene                         |
| 6. 1'-Hydroxy-1',2'-dihydrophytofluene                          | 17. 1,2,1',2'-Tetrahydrolycopene                             |
| 7. 1-Hydroxy-1,2,7',8',11',12'-hexahydrolycopene                | 18. Hydroxyspheroidene (1'-hydroxy-1',2'-dihydrospheroidene) |
| 8. Chloroxanthin  | 19. 1-Methoxy-1,2-dihydrophytoene                            |
| 9. 1-Methoxy-1,2,7',8',11',12'-hexahydrolycopen-4-one           | 20. 1'-Methoxy-1',2'-dihydrophytofluene                      |
| 10. 1-Methoxy-1,2,7',8'-tetrahydrolycopen-4-one                 | 21. 1-Methoxy-1'-hydroxy 1,2,1',2'-tetrahydrophytofluene     |
| 11. 1-Methoxy-1'-hydroxy-1,2,1',2',7',8'-hexahydrolycopen-4-one | 22. 1'-Hydroxy-3,4,1',2',11',12'-hexahydrospheroidene        |
|   | 23. 11',12'-Dihydrospheroidene                               |
|   | 24. 3,4-Dihydrospheroidene                                   |

(b) *Chromatiaceae*

The known distribution of pigments in the Chromatiaceae is given in Table 10.3. The grouping is similar to that previously proposed [4] and is based on the nature of the major pigments present. Organisms in Group 1 which synthesize the normal spirilloxanthin series can be divided into three subgroups: 1A, in which spirilloxanthin (1.19) constitutes more than 80% of the total pigments; 1B, in which it accounts for only 10–30% of the total, the other main pigments being rhodovibrin (10.2) and anhydrorhodovibrin (10.1); and 1C in which synthesis is blocked at the rhodopin (2.16) stage. The major pigment of organisms in Group 2 is okenone (2.8) whilst the characteristic components of Group 3 pigments are cross-conjugated compounds such as rhodopin-20-al (2.21). A pattern of pigmentation similar to that found in Group 1A is also encountered in the Rhodospirillaceae (Table 10.1), but the most important difference between the Rhodospirillaceae and the Chromatiaceae is the presence of okenone in a number of members of the latter. The appearance of okenone suggests a close evolutionary link

Table 10.3 Pigments of the *Chromatiaceae*  
(Pigments italicized are major pigments)

Organism	Pigment	Group	Ref.
<i>Amoebobacter pendens</i>	2, 7, 9, 10	1A	4
<i>Amoebobacter roseus</i>	7, 10	1A	4
<i>Chromatium minus</i>	6	2	4
<i>Chromatium minutissimum</i>	2, 10 <sup>a</sup>	1B	45
<i>Chromatium okenii</i>	6	2	4, 42
<i>Chromatium</i> sp. strain D	1, 2, 7, 8, 9, 10, 11	1B	43
1611	1, 2, 7, 8, 10, 11, 12	1B	4
6411	1, 2, 13, 14, 15	1C	4
6412	1, 2, 8, 10	1B	4
1312	1, 2, 7, 8, 10	1A	4
—	6	2	4
(Aral sea)	1, 2, 7, 10		44
<i>Chromatium violascens</i>	1, 2, 3, 4, 5, 7, 10	1B	4
<i>Chromatium vinosum</i>	1, 2, 7, 9, 10, 13, 16	1B	4, 26
<i>Chromatium warminga</i> 1113	1, 2, 3, 4, 5	3	4
1311	1, 2, 3, 4, 5, ?15	3	4
6512	1, 2, 3, 4, 5	3	4
Melbourne1	1, 2, 3, 4, 5	3	46
Ostrau	1, 2, 3, 4	3	47
<i>Chromatium weissei</i>	6	2	4
<i>Ectothiorhodospira halophila</i>	1, 2, 9, 10	1A	47A
<i>Ectothiorhodospira mobilis</i>	8, 9, 10	1A	47A
<i>Ectothiospira shaposhnikovii</i>	1, 2, 8, 9, 10	1A	47A
<i>Lamprocystis roseopersicina</i>	1, 3, 4, 5, 18, ?19	3	48, 49
<i>Rhodotherce conspicua</i> 6611	6, 10	1A	4
<i>Rhodotherce pendens</i> 1314	1, 6, 10	1A	4
<i>Rhodotherce</i> spp. (Aral sea)	6 (also <i>cis</i> -6)		44
<i>Thiocapsa floridana</i> st. 6311	1, 2, 8, 9, 10	1A	4
1711	10	1A	4
1813	2, 6, 8, 9, 10	1A	4
<i>Thiocapsa pfennigii</i> <sup>b</sup> 6612	1, 2, 7, 8, 9, 10, 20, 21	1A	50, 51
<i>Thiocapsa roseopersicina</i>	1, 2, 7, 8, 9, 10	1A	4, 52, 53
<i>Thiocystis gelatinosa</i> <sup>c</sup>	6, 23, 24, 25, 26,	2	48, 51
<i>Thiocystis</i> sp. (Lascalles)	1, 2, 8, 10	1B	4
1313	1, 2, 3, 4, 5	3	4
2311 <sup>c</sup>	1, 2, 3, 4, 10	3	4
6111	1, 2, 3, 4, 5, 7	3	4, 48
(Eimhjellen)	1, 2, 3, 4, 5	3	54
<i>Thiodictyon</i> spp.	1, 2, 3, 4, 5, 20	3	4, 48
<i>Thiopedia rosea</i> <sup>d</sup>	1, 2, 8, 22	?	55
<i>Thiopedia</i> spp. (Lascalles)	1, 2, 7, 8, 9, 10	1A	4
<i>Thiospirillum jenense</i> 1112	1, 2	1C	4

## Key

- |                   |        |                                  |        |
|-------------------|--------|----------------------------------|--------|
| 1. Lycopene       | (1.11) | 5. 13- <i>cis</i> -lycopen-20-al |        |
| 2. Rhodopin       | (2.16) | 6. Okenone                       | (2.8)  |
| 3. Rhodopin-20-ol | (2.20) | 7. Anhydrorhodovibrin            | (10.1) |
| 4. Rhodopin-20-al | (2.21) | 8. Rhodovibrin                   | (10.2) |

between certain *Chromatium* spp. and the green photosynthetic bacteria because, as will become clear in the next section, these are characterized by the production of aromatic carotenoids. Until recently it was considered that the cross-conjugated pigments rhodopin-20-al and rhodopin-20-ol (2.20) were confined to the Chromatiaceae but it is now known that they exist in *Rps. viridis* and *Rps. acidophila* as indicated in Table 10.1.

### 10.1.2 GREEN PHOTOSYNTHETIC BACTERIA

#### (a) *Chlorobiaceae*

The green photosynthetic bacteria are characterized by the presence of carotenoids with aromatic rings (Table 10.4); it was in these organisms that such pigments were first found outside the animal kingdom (they are present in sponges, see Vol. II). They were considered to be unique to the Chlorobacteriaceae until similar pigments were found in Chromatiaceae (see previous section). Four isolates of a brown Chlorobiaceae (formerly *Phaeobium* sp.) contained isorenieratene (=leprotene) (9.14) a characteristic pigment of some Mycobacteria (see Chapter 9) and some sponges (see Vol. II), and  $\beta$ -isorenieratene (10.5) not previously described in Nature. These represent the major pigments but they are accompanied by much smaller amounts of chlorobactene (1.53) (the characteristic pigment of the Chlorobacteriaceae) and  $\beta$ -carotene (1.13) which is not frequently encountered in photosynthetic bacteria [61].

Key for Table 10.3 cont.

- |  |  |
|--|--|
| 9. Monodemethylated spirilloxanthin (10.3)               | 19. Methoxy 1,2-dihydro-1-lycopen-20-al                |
| 10. Spirilloxanthin (1.19)                               | 20. 3,4,3',4'-Tetrahydrospirilloxanthin                |
| 11. 3,4-Dehydrorhodopin                                  | 21. 3,4,3',4'-Tetrahydrospirilloxanthin-20-al          |
| 12. 1,1'-Dihydroxy 1,2,1',2'-tetrahydrolycopene          | 22. Spheroidenone                                      |
| 13. Neurosporene (2.14)                                  | 23. 1'-Methoxy-1',2'-dihydro- $\gamma$ -caroten-4'-one |
| 14. $\zeta$ -Carotene (1.14)                             | 24. T-484 (Structure not fully elucidated) [51]        |
| 15. Chloroxanthin  | 25. 1-Methoxy-1,2,1',2'-tetrahydro-lycopene            |
| 16. 3,4-Didehydrorhodopin                                | 26. 1-Methoxy-4-oxo-1,2-dihydro-8-apolycopene-8'-al    |
| 17. 1-Methoxy-1'-hydroxy-1,2,1',2'-tetrahydrophytofluene |  |
| 18. 1-Methoxy 1,2-dihydrolycopen-20-ol                   |  |

#### Notes

<sup>a</sup> Lycoxanthin (16-hydroxylycopene) rather unexpectedly reported [60].

<sup>b</sup> Previously described as *Thiococcus* sp. [52].

<sup>c</sup> Also unidentified pigments.

<sup>d</sup> Also a spheroidenone-like pigment.

<sup>e</sup> Formerly *Thiothece*.

Table 10.4 Carotenoids of the *Chlorobacteriaceae*  
(Italicized pigments are major components)

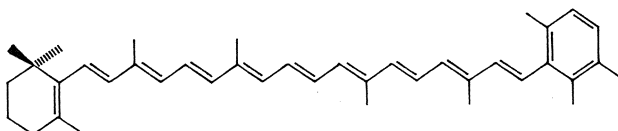
Organism	Pigment	Ref.
<i>Chlorobium limicola</i>	1, 2, 3, 4(?)	56-58
<i>Chlorobium limicola</i> (formerly <i>thiosulfatophilum</i> ) (4 strains)	(a) all strains 3 (b) some strains 1, 2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13	56, 57, 60 59
<i>Chlorobium phaeobacterioides</i>	3, 15, 16, 17	61
<i>Chlorobium phaeovibrioides</i>	3, 15, 16, 17	61
<i>Chlorobium vibrioforme</i> (9 strains)	(a) all strains 3, 5 (b) some strains 1, 2, 4, 6, 7, 8, 9, 10, 11, 12	59
<i>Chloropseudomonas ethylica</i> <sup>a</sup>	2, 3, 4, 5, 10, 14	56
<i>Pelodictyon luteolum</i> (formerly <i>aggregatum</i> ) (2 strains)	(a) both strains 3, 4 (b) one strain 6, 9, 10, 13, 14	

#### Key

- |   |        |                                 |        |
|---|--------|---------------------------------|--------|
| 1. Neurosporene   | (2.14) | 9. Phytoene                     | (1.36) |
| 2. Pro- $\gamma$ -carotene  | (1.55) | 10. Phytofluene                 | (1.37) |
| 3. Chlorobactene  | (1.53) | 11. Hydroxy- $\gamma$ -carotene |        |
| 4. Anhydorrhodovibrin   | (10.1) | 12. Hydroxyneurosporene         |        |
| 5. Hydroxychlorobactene   |        | 13. Hydroxy-P432                |        |
| 6. P-432  |        | 14. $\gamma$ -Carotene          | (1.38) |
| 7. Lycopene   | (1.11) | 15. $\beta$ -Carotene           | (1.13) |
| 8. 1'-Methoxy-3',4'-didehydro-<br>1',2'-dihydro- $\gamma$ -carotene |        | 16. $\beta$ -Isorenieratene     | (10.5) |
|   |        | 17. Isorenieratene (Leptotene)  | (9.14) |

#### Note

<sup>a</sup> Now shown to be a mixed culture of *Chlorobium* or *Prosthecochloris aestuarii* with *Disulfuromonas acetoridans* [56B].



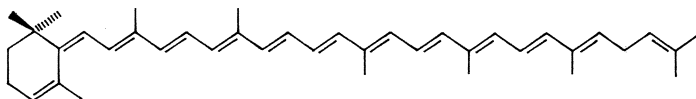
(10.5)  $\beta$ -Isorenieratene

#### (b) *Chloroflexaceae*

A remarkable gliding filamentous photosynthetic organism (*Chloroflexus aurantiacus*) exists in which  $\gamma$ -carotene (1.38) and glycosides of 1'-hydroxy-



1',2'-dihydro- $\gamma$ -carotene and its 3',4'-didehydro and 3',4'-didehydro-4-keto-derivatives together with  $\beta$ -carotene represent by far the major carotenoids present. Much smaller amounts of *retrodehydro*- $\gamma$ -carotene (10.6), 4-keto- $\gamma$ -carotene, and echinenone (4-keto- $\beta$ -carotene) are present [62, 62A]. This distribution makes the taxonomic relationship of this organism with other photosynthetic bacteria very difficult to assess, the only major similarities are the hydration of the 1,2-double bond and the subsequent glycosylation of the 1-hydroxy derivatives.



(10.6) *Retrodehydro*- $\gamma$ -carotene

## 10.2 LOCALIZATION OF CAROTENOIDS

Carotenoids in the photosynthetic bacteria are located in the chromatophores which are part of the membrane system of the bacteria. They are present as well-defined chromoproteins associated with bacteriochlorophyll. Three carotenobacteriochlorophyll protein complexes have been obtained from *Chromatium* [63]; all three contain different light-harvesting forms of chlorophyll but two contain the same carotenoids, spirilloxanthin (1.19) and rhodopin (2.16) in the same ratio, 1:2. In the third complex the ratio of the two pigments is reversed and there is therefore much more carotenoid present relative to the bacteriochlorophyll. Two carotenoprotein complexes have been isolated from *Chromatium minutissimum*, one (B850; B = bulk and 850 indicates the absorption maximum) contains rhodopin whilst the other, B890, contains spirilloxanthin [64]. A complex obtained from *Rsp. rubrum* chromatophores by treatment with sodium dodecyl sulphate (SDS) has a molecular weight of 12 000 and a major absorption maximum at 870 nm; when treated with acetone spirilloxanthin is liberated [65]. Two complexes, B870 and B800–850, have been obtained from *Rps. sphaeroides* [66–68]. The minimal functional unit of the B870 complex contains three molecules of bacteriochlorophyll, one molecule of carotenoid and two molecules of protein (mol. wt. about 10 000 Daltons each) [67]. *In vivo* the complex probably exists as an aggregate. Recent observations on the absorption and circular dichroic spectra of the isolated complex and the intact photosynthetic membrane indicate that the structure of the extracted complex is the same as that *in situ* [68]. The light harvesting complex Bchl II from *Rps. capsulata* contains carotenoids [68A].

Reaction centres (see Chapter 3) have been obtained free from the light harvesting complex from *Rps. sphaeroides* [69–73] and *Rsp. rubrum* [74]. They also contain carotenoids. Difference spectra of reaction centres with and without bound carotenoids [75] and resonance Raman spectroscopy suggest

that *in situ* the carotenoid component is in the 15-*cis* configuration [76, 77]. As analyses of the extracted pigments do not indicate the presence of *cis* carotenoids, these observations may be significant from the functional point of view. Further details of this aspect have been given in a recent review [78].

### 10.3 GENERAL FACTORS CONTROLLING SYNTHESIS

#### 10.3.1 NITROGEN

Leucine, which is extremely carotenogenic in some fungi (see Chapter 8), has little or no effect on pigment production in *Rsp. rubrum* [29].

#### 10.3.2 LIGHT

High light intensity reduces carotenogenesis in photosynthetic bacteria. For example the carotenoid concentration in a green mutant of *Rps. sphaeroides* was maximal at the lowest light intensity (538 lx) examined and five times greater than that in a culture grown under an intensity of 53 800 lx. Because

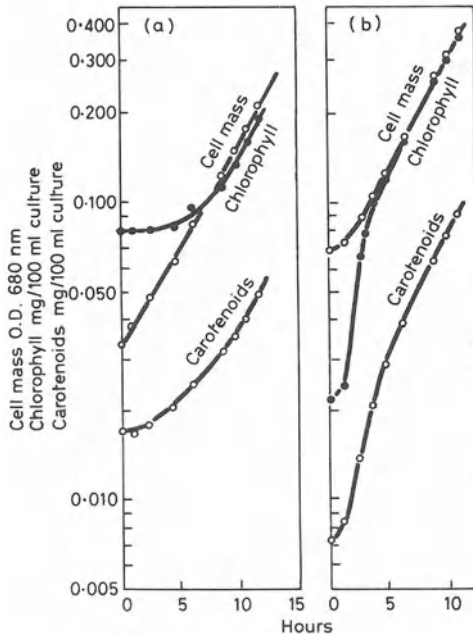


Fig. 10.1. The effect of sudden changes of light intensity on cell mass, chlorophyll and carotenoid synthesis in anaerobic cultures of the green mutant of *Rps. sphaeroides* [79]. (a) Cells previously growing exponentially in dim light (538 lx) exposed at zero time to stronger light (5810 lx); (b) cells previously growing in bright light (53 800 lx) exposed at zero time to 5810 lx.

of this if the intensity of illumination of exponentially growing cells is suddenly increased growth continues at essentially the same rate but carotenoid synthesis is inhibited until the concentration of the pigments falls to the level characteristic of the new light intensity (Fig. 10.1) [79]. These changes are probably due to a primary effect on the synthesis of the photosynthetic membranes (chromatophores) [80–82].

### 10.3.3 OXYGEN

Oxygen has both a qualitative and quantitative effect on some of the photosynthetic bacteria which can tolerate it. If *Rps. spp.* and *Rsp. rubrum* are cultured in a vigorously oxygenated medium then the carotenoid levels are reduced almost to zero [79]; under less vigorous conditions intermediate pigment levels are observed [29]. If a  $N_2$  atmosphere for a culture of *Rps. sphaeroides* is replaced by oxygen then there is an immediate retardation of carotenogenesis, with little if any effect on growth. A return to anaerobiosis results in the pigment production being restored to its original level (Fig. 10.2) [79]. The synthesis in *Rps. sphaeroides* when an aerobic dark culture is taken into the light and put under nitrogen is considered to be only one manifestation of a stimulation of membrane formation [83]. An unexpected

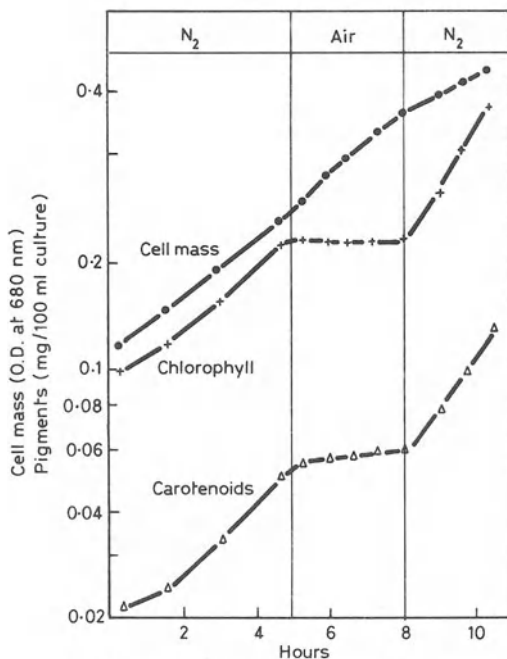


Fig. 10.2. Effect of air on growth, chlorophyll and carotenoid synthesis in wild type *Rps. sphaeroides* cultured under continuous illumination [79].

observation is that demethylated spheroidenone is one major pigment of *Rps. capsulata* grown aerobically in the dark [100] whereas spheroidenone is a major pigment in the light (Table 10.1). Apparently light is necessary for methylation.

#### 10.3.4 INHIBITORS AND STIMULATORS

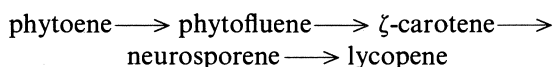
Diphenylamine (DPA) has the same effect on a number of photosynthetic bacteria as on other micro-organisms (Chapters 2, 8, 9), that is, it inhibits the desaturation of phytoene which accumulates in place of the coloured carotenoids in *Rsp. rubrum* [29, 30, 32, 79], *Chromatium* spp. [57, 84] and *Chlorobium* spp. However, DPA has little or no effect on *Rps. sphaeroides*, *Rps. gelatinosa* and *Rps. capsulata* [85] or *Rps. viridis* [25]. 2-Hydroxybiphenyl also inhibits carotenogenesis [86] whilst Simazine and Monolinuron increase the cellular concentration of carotenoids because of their specific inhibitory effect on growth [87]. Nicotine, which inhibits cyclization of acyclic carotenoids in fungi and non-photosynthetic bacteria (Chapters 2, 8, 9) also inhibits the hydration reaction in *Rps. sphaeroides*, *Rps. gelatinosa* and *Rsp. rubrum* and cyclization in *Rm. vannielii*, but has little overall effect on *Rps. viridis* [25]. Similarly in *Rsp. rubrum*, spirilloxanthin synthesis is inhibited by 4-[ $\beta$ -(diethylamino)-ethoxy] benzophenone with the simultaneous accumulation of lycopene at a concentration some 5–6 times greater than that of spirilloxanthin in untreated cultures [88]. The herbicide CPTA inhibits to some extent the hydrogenation of the C-1,2 double bond in *Rps. viridis* [25].

### 10.4 BIOSYNTHESIS

Many experiments indicate putative biosynthetic pathways in photosynthetic micro-organisms but definitive proof for most of the steps is still lacking. The major characteristic reactions of these micro-organisms which must be considered are (i) hydration of the 1,2-double bond with subsequent methylation of the hydroxyl group at C-1; and (ii) insertion of a double bond at C-3,4. Other rather less widespread characteristics which have to be accounted for include (1) hydrogenation of the C-1,2 double bond, (ii) insertion of keto groups in conjugation with the main polyene chain, (iii) the oxidation of in-chain methyl groups and (iv) the formation of aromatic rings. These changes can take place at the lycopene (1.11) or neurosporene (2.14) level.

#### 10.4.1 RHODOSPIRILLACEAE

Inhibition of spirilloxanthin synthesis in *Rsp. rubrum* by diphenylamine (DPA) and the simultaneous accumulation of phytoene [29, 30], indicated that the normal sequential pathway:



was operative as the first stage in spirilloxanthin synthesis. This was confirmed by the fact that spirilloxanthin was synthesized at the expense of phytoene when DPA-inhibited cells were washed free from inhibitor and re-incubated anaerobically [29, 30] in the light; kinetic studies of the conversion also indicated the above pathway [32] as did the observation that lycopene was present in immature normal cells and disappeared as spirilloxanthin accumulated in mature cells [89]. The accumulation of very large amounts of phytoene in DPA-inhibited cells raised problems of interpretation in the early kinetic experiments, but it is reasonable to explain the excessive production by the removal of feed-back control of synthesis when formation of the end product, spirilloxanthin, is inhibited. Recent work has, however, indicated that the conventional phytoene  $\longrightarrow$  lycopene pathway just outlined is not functioning in *Rsp. rubrum* but that is replaced by a modified pathway in which 7,8,11,12-tetrahydrolycopene takes the place of  $\zeta$ -carotene (1.38) in the sequence indicated in Fig. 10.3 [90]. The probable pathway from lycopene to

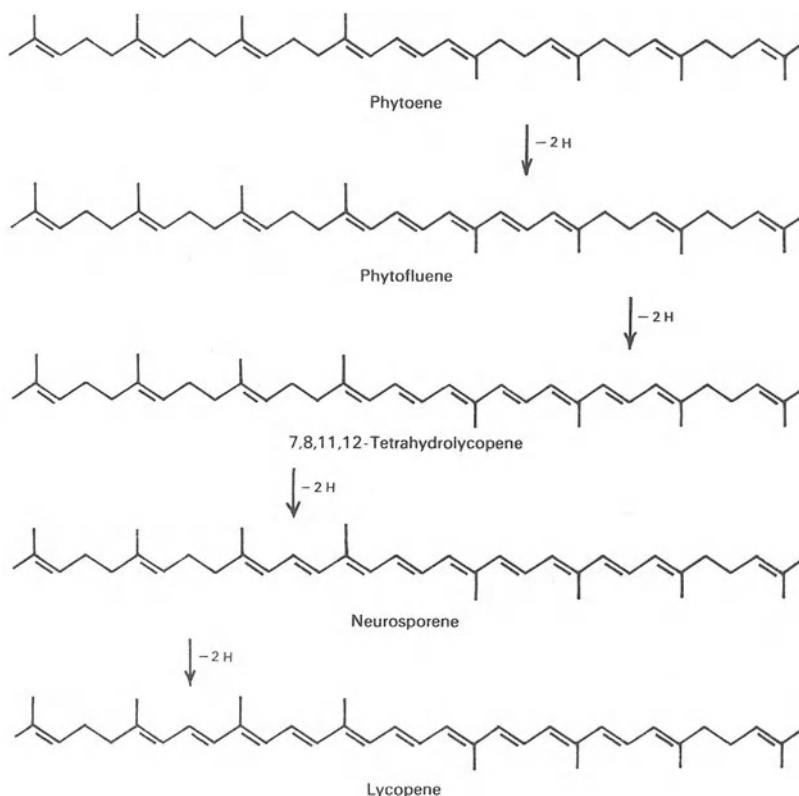


Fig. 10.3. Pathway from phytoene to lycopene in photosynthetic bacteria.

spirilloxanthin would appear to involve hydration at C-1,2, desaturation at C-3,4 and methylation of the hydroxyl group occurring in that order first at one end of the molecule and then at the other end (Fig. 10.4). The hydration, which is only one of a number of ways enzymes can attack the terminal C-1,2 double bond of lycopene (see Chapter 2), is probably carried out as indicated in Fig. 10.5. The methyl groups at C-1 and C-1' arise conventionally by transmethylation via *S*-adenosylmethionine [91–93] (see also Chapter 2).

An alternative pathway to spirilloxanthin has been suggested by a consideration of the pigments present in *Rps. gelatinosa*. But first, the relatively simpler situation in *Rps. sphaeroides* must be considered. This organism synthesizes large amounts of spheroidene (1.41) and hydroxyspheroidene under anaerobic conditions and spheroidenone (1.42) and hydroxyspheroidenone under aerobiosis. These observations led to a logical paper-proposal for spheroidene and hydroxyspheroidene biosynthesis indicated in Fig. 10.6 [2, 3, 94]. This scheme takes in a pathway (A) which starts with attack at the C-1,2 double bond at the neurosporene level of desaturation and not at the lycopene level. Only recently has any experimental support for this pathway to hydroxyspheroidene been obtained [22]. High concentrations of nicotine (10 mM) completely inhibit synthesis of spheroidene and spheroidenone indicating that it acts on the enzyme hydrating the terminal C-1,2 double bonds of an acyclic carotenoid, in this case neurosporene, which accumulates in place of the normal pigments. At lower concentrations of nicotine (up to 5 mM) only one end of the precursor is affected so that although hydroxyspheroidene disappears spheroidene increases by a concomitant amount. In nicotine-inhibited cultures a postulated intermediate, demethylated spheroidene [16] was detected for the first time. Thus pathway A illustrated in Fig. 10.6 now has some biological reality. However 11,12-dihydrospheroidene has been detected in *Rps. sphaeroides* [23] which indicates that hydration of the C-1,2 double bond can occur at the step just prior to the formation of neurosporene, that is at the 7,8,11,12-tetrahydrolycopene step. So an alternative pathway (B, Fig. 10.6) suggests itself [21]. It remains for further experiments to decide whether this is of any major significance *in vivo* or whether 11,12-dihydrospheroidene is a dead-end metabolite, resulting from lack of complete specificity of the hydratase enzyme.

The co-occurrence of spheroidene (1.41) and spirilloxanthin (1.19) in *Rps. gelatinosa* has given rise to the suggestion that, as hinted at earlier, an alternative pathway for spirilloxanthin synthesis exists in this organism (Fig. 10.7). The main evidence for this is negative, that rhodopin (2.16), an intermediate in the normal pathway (Fig. 10.3), is never encountered in *Rps. gelatinosa*. However, kinetic studies support this pathway. Recently the detection of spheroidene [22] and hydroxyspheroidene [22, 23] in diphenylamine-inhibited cultures of *Rsp. rubrum* led to the suggestion that the alternative pathway for spirilloxanthin (Fig. 10.7) also exists side by side with the

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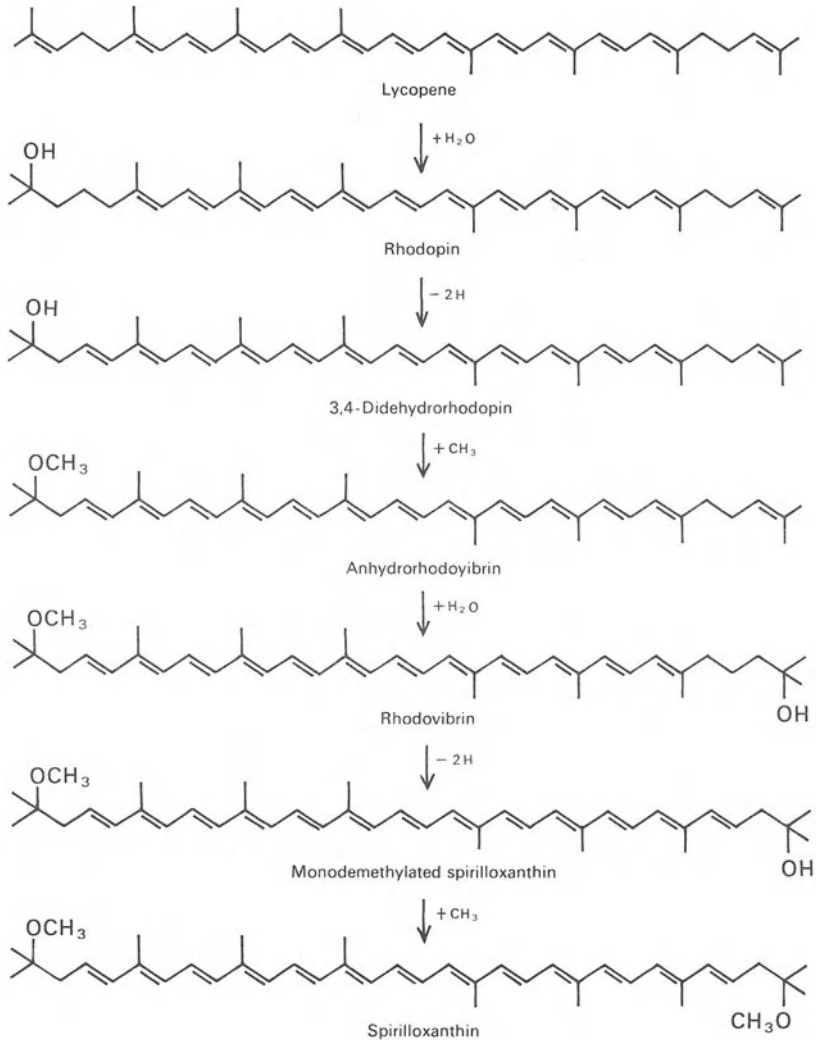


Fig. 10.4. Pathway of spirilloxanthin synthesis from lycopene via rhodopin in *Rsp. rubrum*.

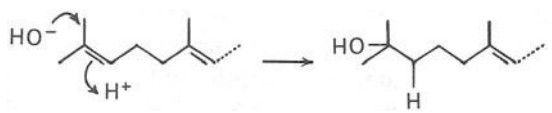


Fig. 10.5. Mechanism of hydration at C-1,2 in rhodopin biosynthesis.

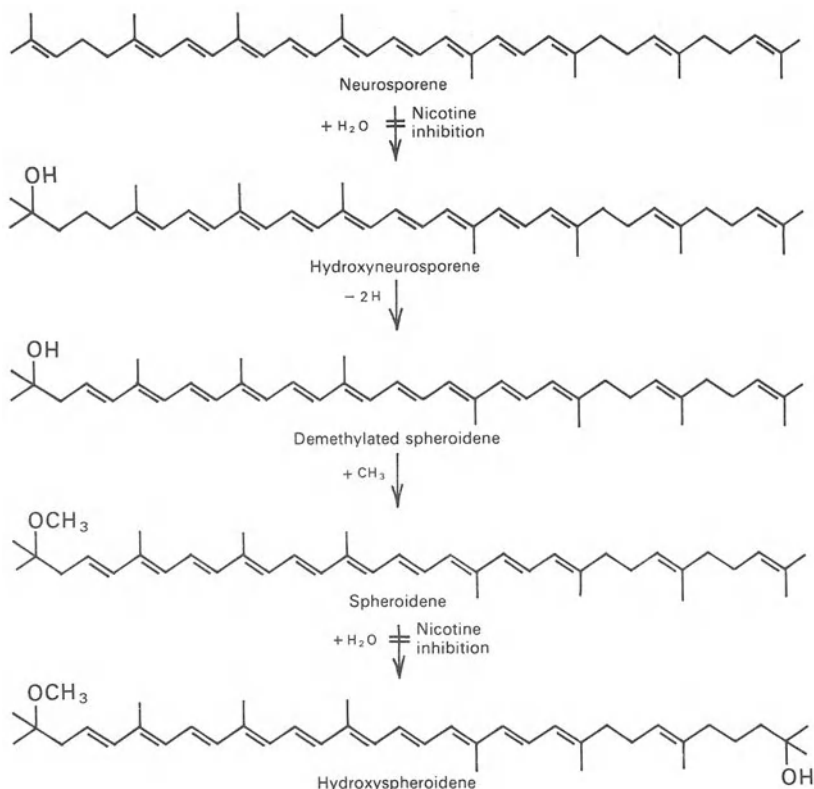
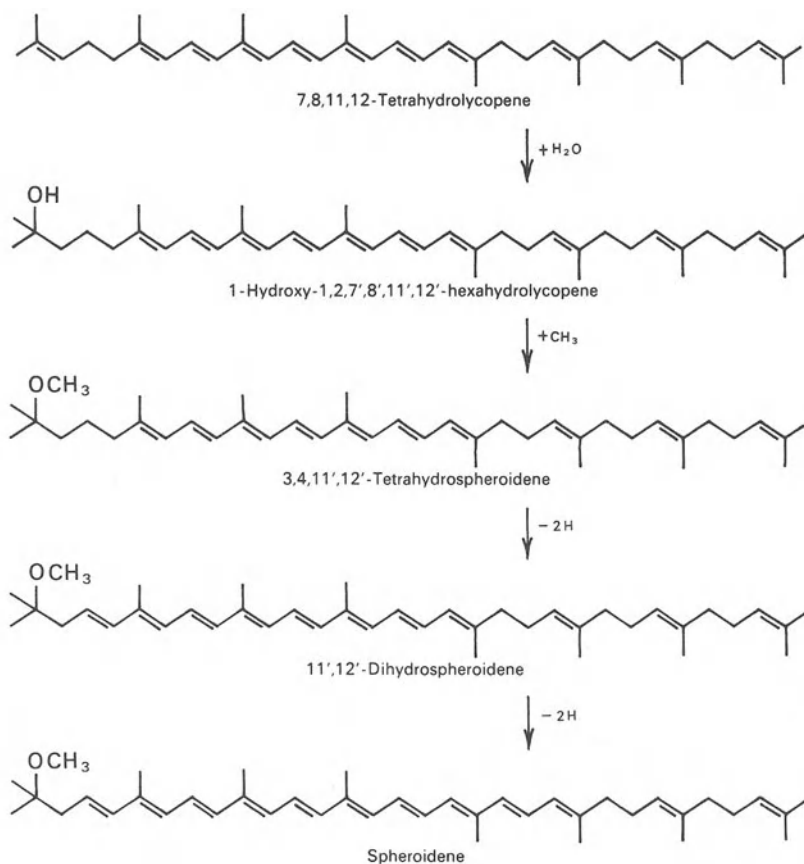


Fig. 10.6. (a) and (b) Alternative pathways of spheroidene and hydroxyspheroidene biosynthesis.

normal pathway (Fig. 10.3) in this organism. However, detailed investigation of the carotenoids in diphenylamine-inhibited cultures of *Rsp. rubrum* revealed the presence of hydroxy and methoxy derivatives of all the early intermediates: phytoene, phytofluene and 7,8,11,12-tetrahydrolycopene [22]. This strongly suggests that under the metabolic restraint imposed by diphenylamine inhibition, the hydrating and methylating enzymes will utilize the partly saturated polyenes as substrates. So caution must be taken in interpreting results on DPA-inhibited cultures and a decision on the reality or otherwise of alternative pathways of spirilloxanthin synthesis in *Rsp. rubrum* must await work on purified enzyme systems. However, as just stated the existence of such a pathway (Fig. 10.7) seems possible in *Rps. sphaeroides* and *Rps. gelatinosa* because in normal cultures of both organisms small amounts of spirilloxanthin occur together with large amounts of hydroxyspheroidene. In *Rps. capsulata*, on the other hand, no spirilloxanthin is formed which





suggests that desaturation of the C-7,8 bond does not occur in this organism. Although the hydration of the terminal double bonds is a very characteristic reaction in the Rhodospirillaceae, a variation is found in *Rps. viridis* [20, 21] in which the C-1,2 double bonds are hydrogenated and the major end products are 1,2-dihydroneurosporene and 1,2-dihydrolycopene; smaller amounts of lycopene and neurosporene and their 1,2,1',2'-tetrahydro derivatives are also found as are the 1,2-dihydro derivatives of phytoene, phytofluene and 7,8,11,12-tetrahydrolycopene [22]. Oddly enough,  $\zeta$ -carotene, an uncommon carotene in photosynthetic bacteria, is present but its dihydro derivative is absent. Studies with inhibitors [24] suggest that the 1,2-dihydro-carotenes are formed from the parent carotenes lycopene and neurosporene. Clearly yet another mechanism exists for attack at the terminal double bond without leading to a ring system; it can be formally indicated as in Fig. 10.8 (see also Chapter 2).

In *Rm. vannielii* the fascinating situation exists in which two types of

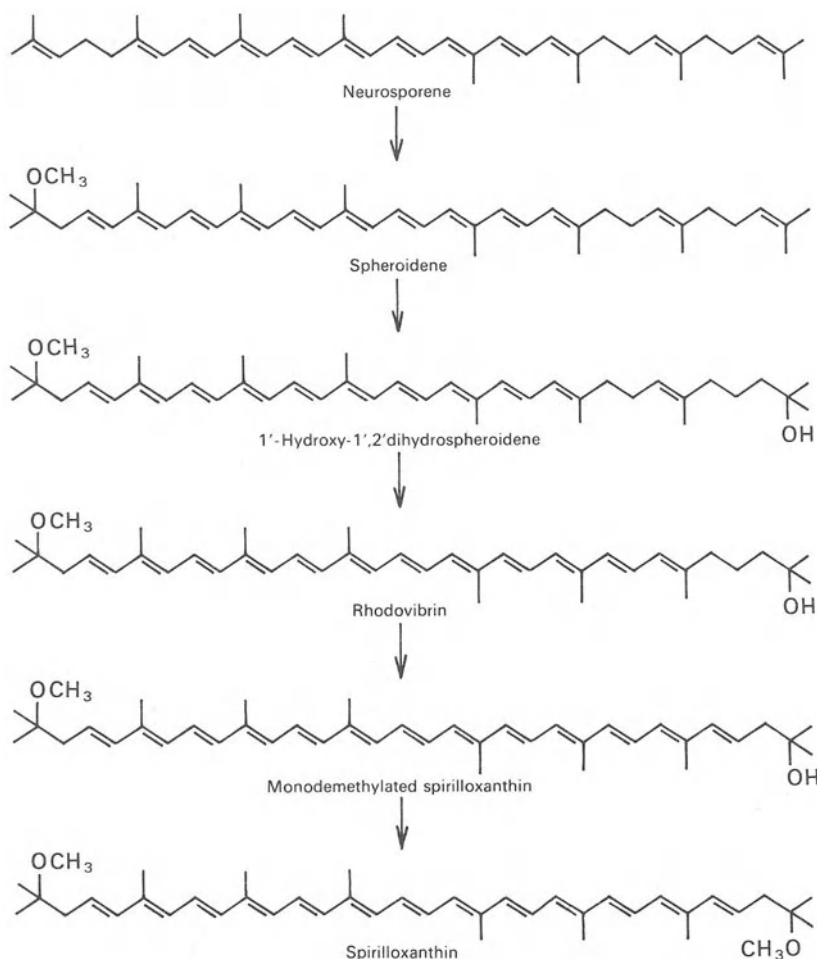


Fig. 10.7 Alternative pathway of spirilloxanthin biosynthesis via spheroidene in *Rps. gelatinosa*.

terminal reactions are taking place simultaneously: hydration, with the production of rhodopin (2.16), and cyclization to form  $\beta$ -carotene (1.13). In this organism both nicotine and CPTA [2-(4-chlorophenylthio) triethyl ammonium chloride] exhibit their inhibitory effect on hydration and on cyclization, so that at an appropriate concentration of either inhibitor synthesis of rhodopin and its derivatives and of  $\beta$ -carotene is almost totally inhibited. They are replaced by an equivalent amount of lycopene and on removal of the inhibitor lycopene is converted into rhodopin. By allowing this conversion to be carried out in the presence of  $D_2O$  one atom of deuterium is incorporated into one molecule of rhodopin (G. Britton *et al.*, unpublished work) (see also Chapter 2).

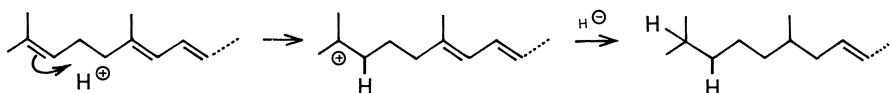


Fig. 10.8. Proposed mechanism for the synthesis of 1,2-dihydroderivatives in *Rps. viridis*.

Other interesting aspects of biosynthesis observed in *Rm. vannielii* are the existence of a 'mixed' carotenoid, with one terminal  $\beta$ -ring and one acyclic end with its terminal C-1,2 group hydrated, and of  $\beta$ -cryptoxanthin (1.60). The appearance of the latter is particularly interesting because the 3-hydroxy substituent is unique amongst carotenoids of photosynthetic bacteria and must, in this instance, be inserted anaerobically. In higher plants and algae it is inserted directly, probably by a mixed function oxygenase (Chapter 2).

There also exist amongst the Rhodospirillaceae other organisms which do not carry a complete set of enzymes to allow the biosynthesis of spirilloxanthin to take place. For example, *Thiospirillum jenense*, *Rsp. molischianum* and *Rsp. fulvum*, produce mainly lycopene (1.11) and rhodopin (2.16) [52].

#### 10.4.2 CHROMATIACEAE

As already indicated, the Chromatiaceae can be divided into three main groups according to whether the carotenoid biosynthetic pathway results in the formation of (i) spirilloxanthin (1.19); (ii) okenone (2.8) and its derivatives or (iii) the cross-conjugated rhodopin-20-al and its derivatives. The spirilloxanthin pathway has been discussed in the previous section and virtually nothing is known about the other pathways so that speculation, based merely on the chemical structure of minor components, is not particularly rewarding at present. Possible pathways to okenone and related keto-carotenoids are indicated in Fig. 10.9. All the compounds suggested as intermediates occur naturally.

#### 10.4.3 CHLOROBACEAE

The main biosynthetic characteristic of the carotenoids of these bacteria is the appearance of an aromatic ring. Only one experimental investigation has been reported on the origin of the aromatic ring of chlorobactene [17]. In *Chloropseudomonas ethylica* the ring is mevalonate-derived and the methyl group at C-2 arises by the specific migration of one of the two methyls at C-1 of a non-aromatic precursor, probably  $\beta$ -carotene. These methyls derive from C-2 and C-3' of mevalonic acid and it is the latter which migrates [87]. Whilst the green members of this Family produce pigments such as chlorobactene with only one aromatic ring, the pigments of the brown members contain two aromatic rings. A 'structural' biosynthetic pathway for these compounds is



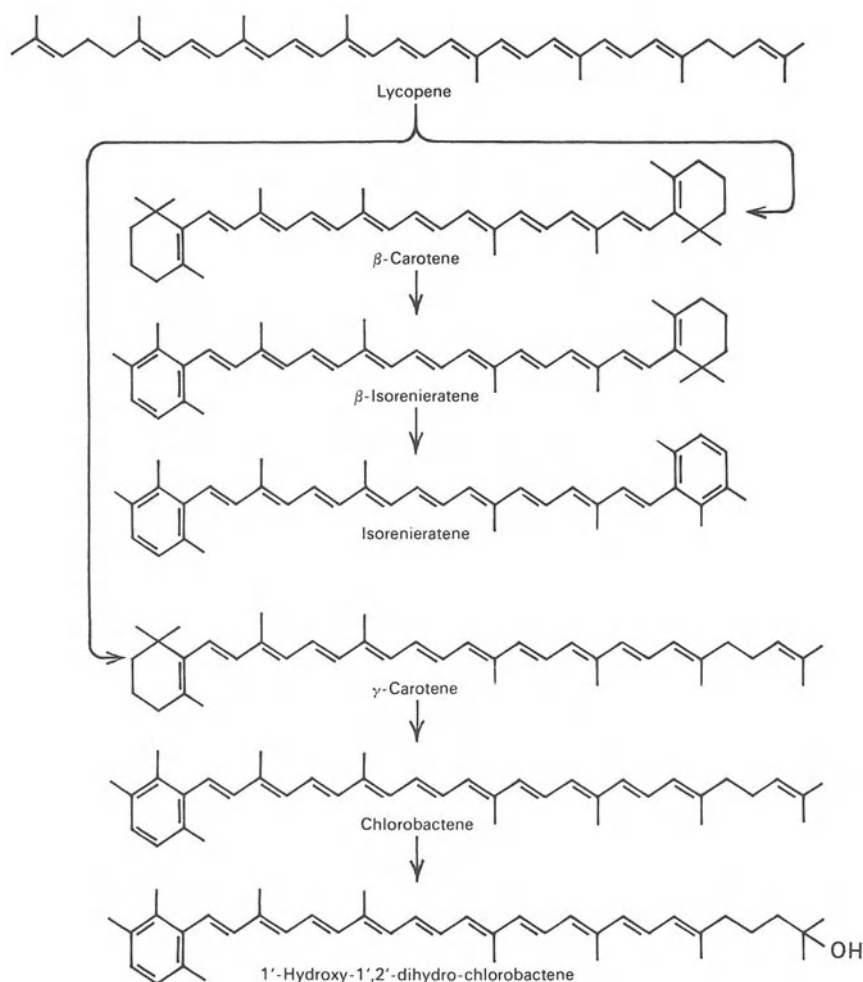


Fig. 10.10. Proposed pathway for synthesis of aromatic carotenoids in the Chlorobiaceae.

indicated in Fig. 10.10 [26]. The biosynthetic pathway to  $\beta$ - and  $\gamma$ -carotenes in *Chloroflexus* probably follows the conventional pathway [26].

#### 10.4.4 INSERTION OF OXYGEN

The hydration of the terminal double bonds in the synthesis of typical carotenoids of photosynthetic bacteria has already been considered. Now the reactions which involve an oxidation step must be discussed. The most spectacular reaction is that which causes the impressive colour change when anaerobic cultures of *Rps. sphaeroides* are allowed access to oxygen. This is

due to the insertion of a ketone group in conjugation with the polyene chain of spheroidene (1.41) and hydroxyspheroidene (yellow pigments) to form spheroidenone (1.42) and hydroxyspheroidenone (red pigments) [12, 17, 79]. In addition spirilloxanthin (1.19), a minor component, is converted into 2,2'-diketospirilloxanthin in *Rps. sphaeroides* [79] and in *Rps. gelatinosa* [12]. These oxidations occur extremely quickly and no intermediates have yet been detected. The source of the oxygen in the keto-group is O<sub>2</sub> [97] and one cell-free system carrying out the reaction has been reported [98]. There is a claim that merely gassing with oxygen-free CO<sub>2</sub> will initiate the conversion, although oxygen-free N<sub>2</sub> will not [99]. The conversion is normally so rapid that one wonders whether traces of O<sub>2</sub> were indeed present in the CO<sub>2</sub> used; on the other hand it is further claimed that the CO<sub>2</sub>-stimulation is inhibited by chloramphenicol whereas the O<sub>2</sub> reaction is not.

Even more interesting is the appearance of keto groups (okenone) (2.8) and oxidized cross-conjugated carotenoids (rhodopinal, rhodopinol) in strict anaerobes. Facultative anaerobic strains of *Rps. acidophila*, for instance, do not form cross-conjugated carotenoids. Nothing is known of the mechanisms involved but a possibility for anaerobic ketone production at C-4 has been suggested (Fig. 10.11). It will be recalled that insertion of a keto group in organisms grown aerobically occurs at C-2 (e.g. spheroidenone) (1.42).

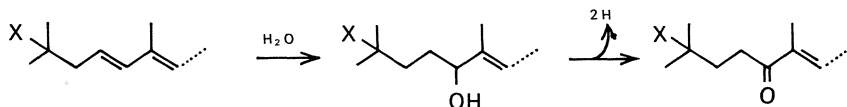


Fig. 10.11. A mechanism for the introduction of carbonyl groups at C-4 in anaerobic photosynthetic bacteria.

On the basis of the cross-conjugated carotenoids detected in various organisms a biosynthetic pathway has been suggested [49]. No experimental evidence is yet available to support this proposal.

#### 10.4.5 FORMATION OF GLYCOSIDES

The glycosides of  $\gamma$ -carotene derivatives in *Chloroflexus* are probably formed by direct transfer of glucose to the parent aglycone as indicated in Fig. 10.12 [26], although no evidence exists to support this plausible view. Rhodopin and rhodopinal glycosides are probably formed in the same way in *Rps. acidophila*.

### 10.5 REFERENCES

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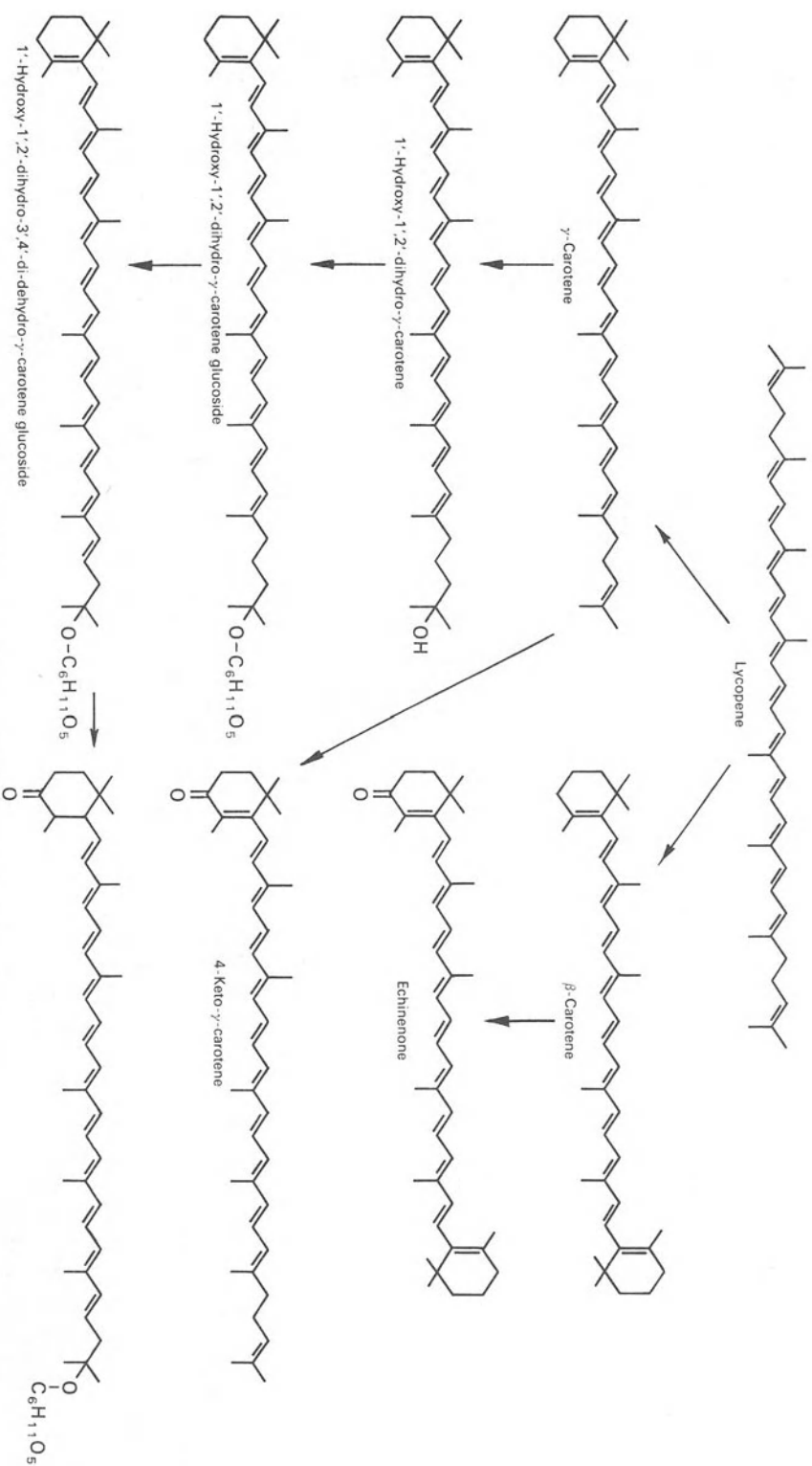


Fig. 10.12. Carotenoid glucoside formation in *Chloroflexus*.

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## [ II ]

# BIOGEOCHEMISTRY OF CAROTENOIDS

### 11.1 INTRODUCTION

In lakes and oceans the overlying water column contains particulate matter, *seston*, which is made up mainly of phytoplankton and zooplankton. Considerable amounts of carotenoids, present in all algae and photosynthetic bacteria, some non-photosynthetic bacteria and some fungi as well as in some zooplankton (see Vol. II), will, therefore, be deposited on the lake or ocean bed. Thus, apart from possible microbial synthesis of carotenoids in the superficial layers of the deposits [1], any carotenoids found in sediments must have survived many thousands of years. The fact that they are found in such materials is ascribed to the special conditions of the environment: (i) lack of oxygen, (ii) lack of light and (iii) low temperature [2]; combined with these conditions is water-insolubility of the pigments.

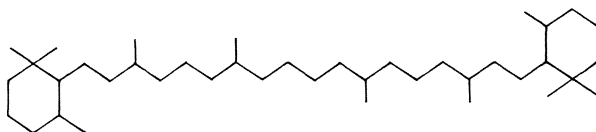
There are many reports of carotenoids in seston [3–8] and it is important to note that the ratio of xanthophylls to carotenes varied, in cases where such measurements were made, from 7:1 to 14:1 [2].

### 11.2 FRESH WATER DEPOSITS

Carotenoids were first encountered in a lake deposit in Russia and subsequently in North Florida in water less than 30 cm deep [10]. Of two types of peat-like deposits from a site near Stockholm, the red and green samples, which were of algal origin, contained only carotenes whilst the detritus deposit contained only xanthophylls [11]. Xanthophylls also predominated in river-borne detritus in an underground pool, whilst, not unexpectedly, air-borne detritus in similar locations, e.g. in a drip pool unconnected with a river, contained little or no pigment [12]. Other sediments which contain carotenoids include Russian marsh sapropels [13, 14], Muscovite lakes [15], Minnesota lakes [16], Californian lakes [17], Lake Washington [18], Swiss lakes [19], Esthwaite Water (UK) [20] and Swedish interglacial gyttja [21] and young Florida mud [22]. It is interesting that the presence of spirilloxanthin (1.19) in

red muds from Kenya [23] and in young California, Mississippi and Mexican muds was due to the fact that the muds were a natural mass culture of purple-sulphur bacteria [25]; the levels in the last three being 27, 222 and 393 mg/kg organic carbon respectively [24]. In the case of the Californian Searles Lake,  $\alpha$ - and  $\beta$ -carotenes (1.12, 1.13) were clearly identified in sediment at least 20 000 years old [17]; in a bog lake their maximum concentration was in the 6000–5000 years old region [26]. *Cis*-isomers are extremely low in these sediments, lower than expected from a normal chemical equilibrium.

Perhydrocarotene (11.1) is present in green river shale laid down  $50 \times 10^6$  years ago in shallow lakes [27, 28]. The relative contribution of land and aquatic plants to such muds is not known.



(11.1) Perhydrocarotene

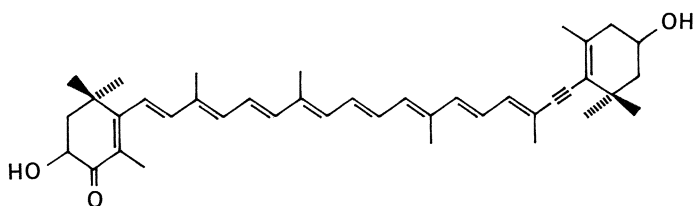
Soil organic matter contains lutein (1.74) and traces of  $\beta$ -carotene (1.13) [29] whilst the fossil plant pigments from bituminous Bulgarian clays are mainly  $\beta$ -carotene and myxoxanthophyll (1.20) indicating their origin from blue-green algae [30]. Similarly the concentration of myxoxanthophyll in lake sediments of several Swiss lakes is closely related to known concentrations of blue-green algae in the lakes in the recent past. [Myxoxanthophyll is a characteristic xanthophyll of many blue-green algae, see Chapter 7.] Studies on the vertical distribution of oscillaxanthin (7.18) a characteristic pigment of *Oscillatoria* spp. (see Chapter 7), in sediments of Lake Washington reflect recent fluctuation in these species since sewage enrichment during the early 1950s [28].

It is frequently stated that carotenes preponderate over xanthophylls in lake sediments and this is probably so, although it may not be so marked as thought because of earlier analytical problems [24]. However, the apparent preferential destruction of xanthophylls still requires explanation because the assumption that xanthophylls are more prone to attack by oxygen ignores the anaerobic environment of the deposits. The calculated half-life for lutein destruction in lake deposits is 16 000 years [20] and it may be that the carotene:xanthophyll ratio of plants contributing to the sediment was different from that encountered nowadays.

### 11.3 OCEAN DEPOSITS

Not a great deal has been reported about ocean muds since the pioneering work of Fox and his colleagues [31–34]. Again, as in freshwater sediments it was found that although xanthophylls predominated in living organisms,

carotenes preponderated in the ocean muds and the following were tentatively identified, rhodopurpurin [lycopene (1.11)], flavorrhodin [neurosporene (2.14)], torulene (2.24), fucoxanthin (1.57), petaloxanthin [mixture of zeaxanthin (1.18), lutein (1.74) and, possibly, antheraxanthin (1.24)], zeaxanthin (1.18), sulcatoxanthin [peridinin (1.71)], antheraxanthin (1.24), glycymerin [pectenolone (11.2)] and leprotene [isorenieratene] (9.14). (The probable modern identifications are given in square brackets.) It is surprising that fucoxanthin and astaxanthin, the main components of phyto- and zooplankton, respectively, are not major components of muds, but contributions of pigments from red yeasts (torulene), Pyrrophyta (peridinin) and photosynthetic bacteria (lycopene and leprotene – which might also arise from sponges, see Vol. II) are clearly indicated.



(11.2) Pectenolone

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