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CAROTENOIDS — A CHEMOSYSTEMATIC APPROACH

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Abstract - The principles of taxonomy, chemotaxonomy and chemosystematics are outlined. Chemosystematics may serve to improve existing classification of organisms by revealing phenetic and phylogenetic relationships. Secondary metabolites hitherto studied for chemosystematic purpose are mentioned. Additional knowledge of biosynthetic pathways leading to the terminal products is a better proof of phylogeny and differentiation. For microorganisms with restricted morphological character, chemosystematics forms an important taxonomic tool.
Properties of carotenoids which favour their use as chemosystematic markers are discussed, and the carotenoids of photosynthetic and other bacteria, yeasts and other fungi, algae and higher plants are discussed separately, in light of available structural evidence and known or postulated biosynthetic pathways.
Systematic changes in structural features of the terminal carotenoid products correlate well with commonly accepted phylogenetic trees. Particularly within photosynthetic bacteria, algae, yeasts and fungi carotenoids are considered a useful tool in classification. In lower animals carotenoid analysis may demonstrate food chain relationship and specific metabolic capacity.
Future prospects for use of carotenoids in classification are considered.

TAXONOMY AND CHEMOSYSTEMATICS

Since long before the times of Linnaeus (1) and Darwin (2) the human race has realized the practical importance and challenging task of organizing or classifying living matter in our environment.
In modern plant classification the classes form a nested hierarchy where a single taxon (group) can only belong to one next higher taxon in the hierarchy. Attempts are made to develop phylogenetic schemes, reflecting the evolution of organisms (3,4).
Taxonomy in the strict sense deals with the principles, procedures and rules of classification (5). A taxonomic system should reflect the totality of similarities and differences in homologous characters of organisms. However, traditionally morphology has been the major source of information on which taxonomic schemes are based, with some contribution from anatomy, cytology, serology, ecology and genetics.
Chemotaxonomy aims at a correlation of chemical evidence with systematic classification based on morphological characters (6). Although morphological characters have a chemical foundation, chemical characters frequently do not have a morphological expression.
In recent years the term chemosystematics has been preferred for studies of chemical relationship between organisms (7). Chemosystematics may serve to a) improve existing classification by revealing phenetic (common features) relationships and b) throw light on phylogenetic (evolutionary) relationships.
The chemical approach to plant classification has mainly been based on micromolecular chemistry of secondary metabolites. In contrast to the ubiquitous low-molecular primary products, the usefulness of secondary metabolites as chemosystematic markers depends on a discontinuous distribution. Examples of such secondary metabolites that have been used are non-protein amino acids (7), alkaloids (6,8), flavanoids (9), betalains (10,11), terpenoids (6,12), steroids (13), polyacetylenes (14,15) and glucosinolates (16), Fig. 1. Many

of these compounds appeal to the human senses (odour, colour, taste) and have therefore attracted attention. Biofunctional evidence increases the knowledge of a chemical character. However, the true function of many of these secondary metabolites may not be established, but they are likely to be useful in some way for the eco-system of the organism.

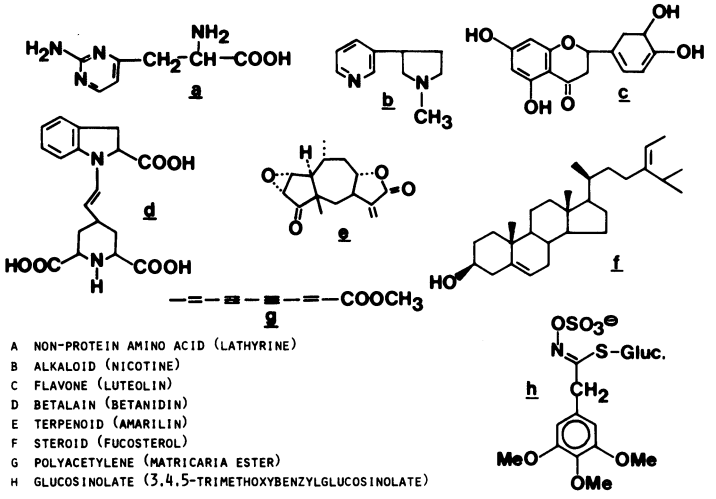


Fig. 1. Examples of compounds belonging to classes of chemosystematically useful secondary metabolites.

In recent years chemosystematic studies have been extended to macromolecules such as proteins and nucleic acids, which in principle carry the information of genes and could reflect more evolutionary history. Particularly the amino acid sequences of cytochrome c (17) and ferredoxin (18) and DNA base ratios (19) have been studied. Although work along these lines is at an early stage future progress is expected.

However, it should be realized that the biosyntheses of secondary metabolites are also a result of the genetic equipment of the organism, reflecting information contained in the semantides (information-carrying molecules such as DNA) (20).

Refined and efficient techniques are now available for the structural elucidation of low-molecular natural products on a micro scale, and explosive activity in this field has occurred during the last 15 years, particularly related to higher plants (21). More recently marine sources have attracted considerable interest (22).

It is known that various secondary metabolites may be synthesized via more than one biosynthetic route, e.g. in the quinolizidine alkaloid series (23,24). Knowledge of biosynthetic pathways is consequently a better historical proof of phylogeny and differentiation than the mere knowledge of specific structures of biosynthetic end products (25,26). Biosynthetic pathways are being increasingly understood in terms of chemical reaction mechanism, and organic chemists have successfully predicted the biosynthesis of several secondary metabolites (26), frequently guided by knowledge of absolute configuration of the products concerned. Biosynthetic studies with isotopically labelled substrates have become important. However, for most secondary metabolites biosynthetic pathways on the enzymatic level have so far been only partly characterized.

For microorganisms like bacteria and unicellular algae morphological characters are much less differentiating than for macroscopic algae and higher plants. Physiological studies, elucidation of energy sources and metabolic pathways with the use of labelled substrates and on the enzymatic level, serological studies, and chemical investigation including DNA base ratios, cell wall constituents, secondary metabolites like pigments etc. are indispensible for classification purposes (27,28). Chemosystematics is here of unique importance as a taxonomic tool.

CAROTENOIDS AS CHEMOSYSTEMATIC MARKERS

With this general background we may ask the question: Are carotenoids chemosystematically useful compounds? Properties in their favour are:

Structural diversity:	Secondary metabolites of sufficient structural variation; ca. 400 structures (29).
Distribution:	Present in all photosynthetic organisms; occasional occurrence in other bacteria, yeasts and fungi.
Biosynthetic pathway:	Common gross biosynthetic route to C_{40}-level with specific terminal steps.
Function:	Many well-established functions; essential for the survival of photosynthetic organisms.
Analysis:	Pigments, readily detected. Quantitative analysis on µg scale easily effected (TLC, HPLC, UV/vis, MS, ^{1}H NMR).

Pioneering attempts by Goodwin (30) to use carotenoids as chemosystematic markers were hampered by insufficient information on all points above. Scattered chemosystematic contributions on carotenoids from higher plants (31,32), algae (33-41), yeasts (42), fungi (43), photosynthetic (44) and other bacteria (45-47) have since appeared.
After a decennium with large progress in elucidation of new structures (48,49) all major carotenoids now appear to be structurally fully characterized (29), and a decrease in novel structural features is noted. However, information on the occurrence of natural carotenoids is steadily increasing. At this stage a general evaluation of carotenoids as chemosystematic markers seems appropriate, especially since carotenoids have not been fully recognized as taxonomically useful cell constituents (6).
The following discussion will be selective in the choice of examples and subjective in evaluation. We shall start our consideration with bacteria, then yeasts, other fungi, algae, higher plants and animals, followed by a conclusion.

Phototropic bacteria

All species hitherto available in pure culture have been subjected to modern carotenoid analysis. The characteristic structural features of the around 85

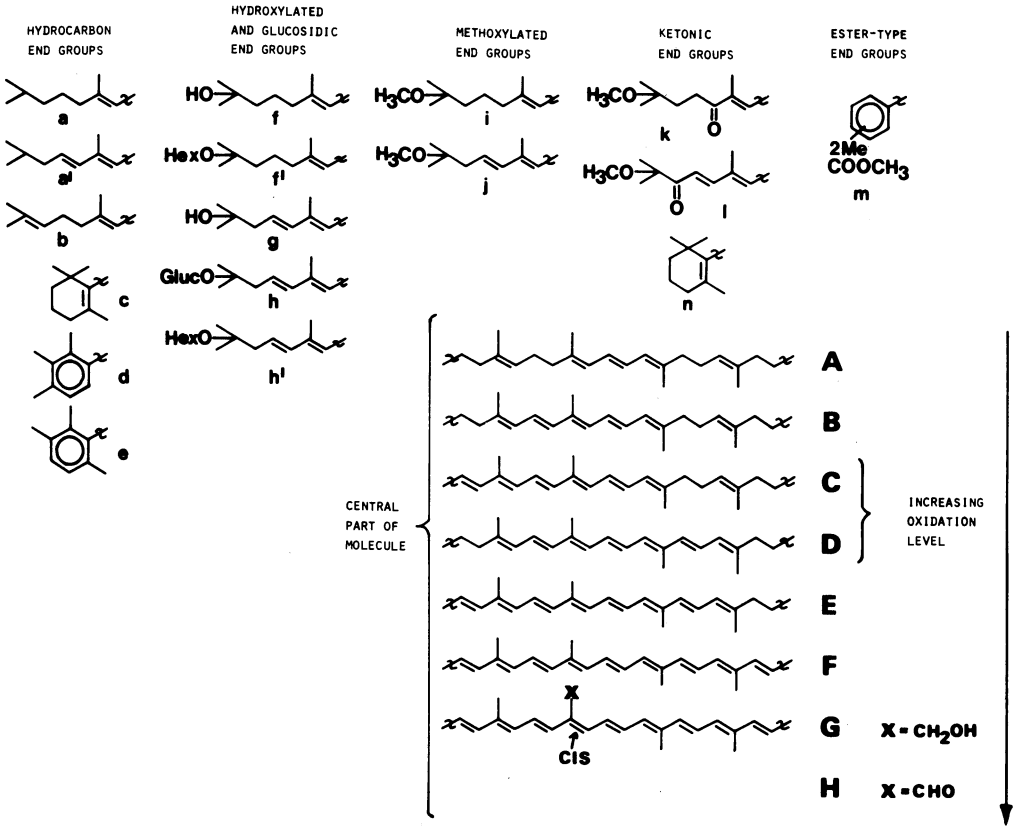


Fig. 2. Structural features of carotenoids from photosynthetic bacteria (50).

different carotenoids encountered (see Fig. 2, taken from a recent review (50)), most frequently comprise aliphatic skeletons with varying polyene chain, carrying tertiary hydroxy or methoxy groups, occasionally tertiary glucosyl substituents. Conjugated carbonyl groups are located at C-2, C-4 or C-20 positions. Cyclic end groups are usually aromatic (Φ- or χ-type). Biosynthetic routes have been proposed mainly from kinetic and structural evidence (51,52,44). At least 12 simple chemical step reactions are required to account for the carotenoids formed. With reference to Fig. 2 these include 1) bisallylic dehydrogenation e.g. A-B, 2) allylic dehydrogenation e.g. f-g, 3) hydration b-f, 4) methylation e.g. g-j, 5) glucosidation g-h, 6) aerobic 2-keto formation j-l, 7) anaerobic 4-keto formation i-k, 8) anaerobic aldehyde formation F-(G?)-H, 9) cyclization to β-ring b-n, 10) aromatization to Φ end-group n-d, 11) aromatization to χ end-group n-e and 12) hydrogenation b-a.
The "half molecule model" (53) is convenient when considering the biosynthesis of the individual carotenoids. In the biosynthetic schemes the sequence of the different step reactions may vary. The four main biosynthetic pathways involved have recently been discussed by Schmidt (44). The most characteristic biosynthetic capacity of each pathway and the number of species producing carotenoids belonging to each pathway are cited in Table 1.

TABLE 1. Biosynthetic pathways for carotenoids of photosynthetic bacteria, adopted from Schmidt (44).

Biosynthetic pathway	Characteristic capacity	Number of species examined			
		Rhodospirillaceae	Chromatiaceae	Chlorobiaceae	Chloroflexus
1. Spirilloxanthin pathway					
i) Normal spirilloxanthin series	$-OCH_3$	8	10		
ii) Rhodopinal series	20-al, $-OCH_3$	4	7		
2. Spheroidene/spheroidenone pathway	2-keto, $-OCH_3$	3			
3. Okenone pathway					
i) Okenone	χ,4-keto, $-OCH_3$		5		
ii) Aliphatic 4-keto-carotenoids	4-keto, $-OCH_3$	1			
4. Isorenieratene pathway					
i) Isorenieratene	Φ			2	
ii) Chlorobactene	Φ			6	
iii) β,γ- and β,β-carotene	Alicyclic				1

Chemosystematically significant conclusions, taking all types of carotenoid-containing organisms into consideration are: 1) Carotenoids with tertiary methoxy groups are restricted to red, phototropic bacteria; not yet encountered in Chlorobiaceae or Chloroflexus. 2) The rhodopinal series is confined to strictly anaerobic phototropic bacteria within Rhodospirillaceae and Chromatiaceae. 3) The spheroidene-spheroidenone pathway is restricted to three Rhodopseudomonas spp. 4) Amongst photosynthetic bacteria aryl carotenoids are restricted to sulphur bacteria: χ end group typical of okenone encountered in Chromatiaceae and Φ end groups in carotenoids of the Chlorobiaceae. Together with other criteria such as sulphur reduction the results suggest possible differentiation in most cases on the basis of the carotenoid complement to family and even genus level. Differences between phenetically related species may be considerable regarding the extent to which transformation to the terminal carotenoid products of the actual pathway is effected. In wider context the lack of chiral centers (disregarding glucose moieties) of the carotenoids of phototropic bacteria is noteworthy.

Other bacteria

Of the more scattered information regarding carotenoids in other types of bacteria, including aryl (54), phenolic (55) and glycosidic (56,57) carotenoids

common C_{40}-xanthophylls (53) etc., attention is drawn to the new series of triterpenoid carotenoids with C_{30} skeletons studied by Davies' group (58,59), Fig. 3. These unique diapocarotenoids call for a new biosynthetic pathway from farnesylpyrophosphate. So far they are encountered in Streptomyces faecium (58,59), Staphylococcus aureus (60) and Halobacterium cuticurubrum (diapophytoene) (61) of remote systematic position.

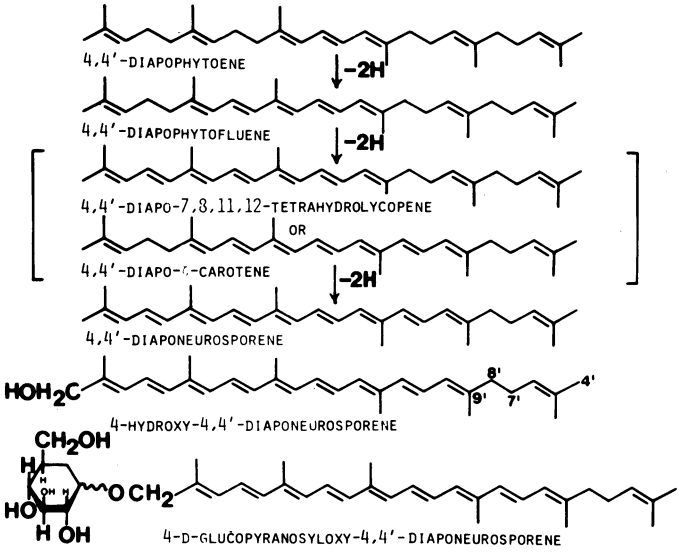


Fig. 3. Bacterial diapocarotenoids (C_{30}).

The other unique class of carotenoids with C_{50} and C_{45} skeletons, Fig. 4, comprises 2(2')-isopentenylated carotenoids with decaprenoxanthin (62), sarcinaxanthin (63), C.p. 450 (64), bacterioruberin (65), dehydrogenans-P452 (66) and 2-isopentenyl-rhodopin (67) as characteristic examples. Such higher carotenoids are hitherto encountered amongst members of the genera Flavobacterium, Halobacterium, Corynebacterium and Sarcina, a distribution pattern that correlates little with present taxonomy. Attempts to correlate guanine + cytosine ratios with biosynthetic routes to C_{30} and C_{50} carotenoids have been made (58).

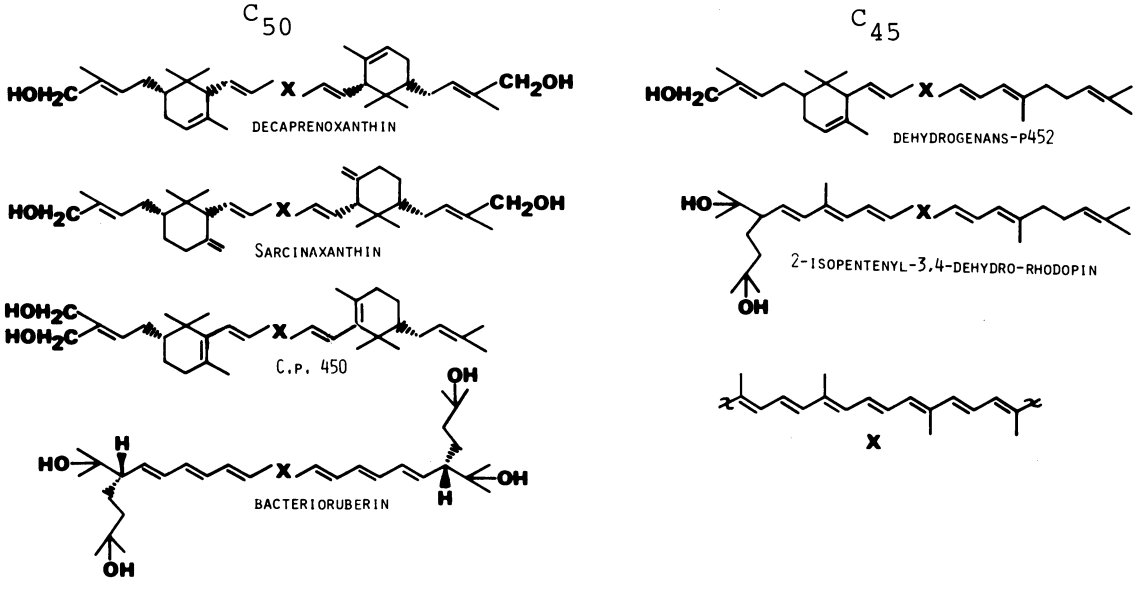


Fig. 4. Examples of C_{50} and C_{45} carotenoids.

Systematic studies within the gliding bacteria by Reichenbach and Kleinig (46,67) reveal a common occurrence of monocyclic C_{40} carotenoids with tertiary monoacyl-O-glucosyl or tertiary O-rhamnosyl groups, Fig. 5. They claim a clear differentiation between the Myxobacteria and gliding bacteria of the Flexibacter-Cytophaga groups in which flexirubin-type pigments are found.

These pigments are esterified non-isoprenoid arylpolyene carboxylic acids (69). However, presence of monocylic C_{40} carotenoid acylated glucosides in a Herpetosiphon sp. (Cytophagaceae) (70) and monocyclic carotenoids in bacteria classified as Saprospira (71) and Flexibacter spp. (72) argues against a clear differentiation. The bromosubstituted aryl-polyenes encountered in the genus Xanthomonas (73,74) which are structurally related to the flexirubin pigments, further demonstrate the need for caution in claiming restricted distribution.

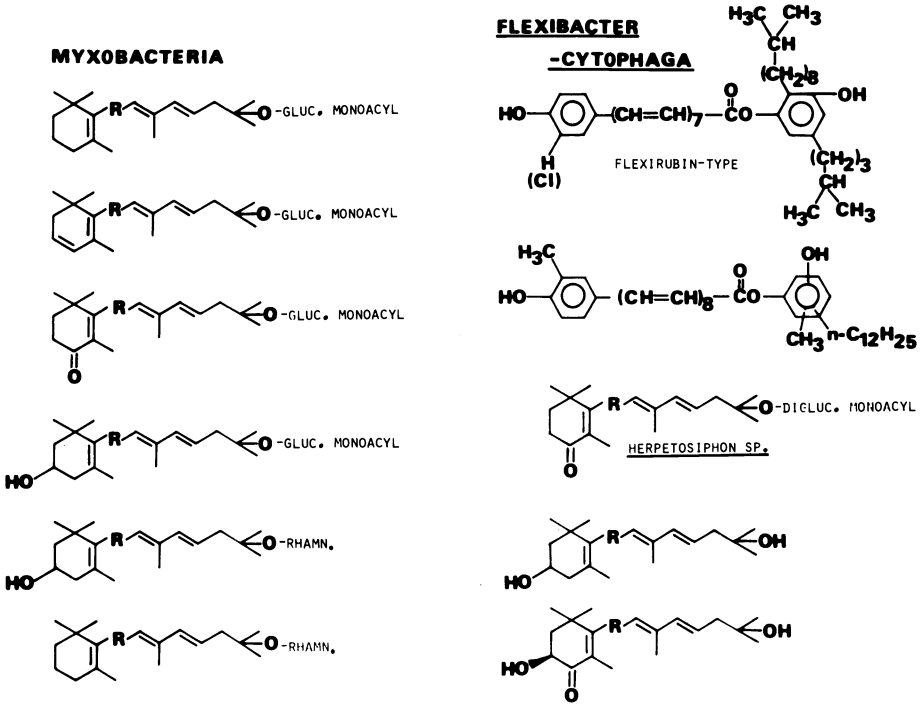


Fig. 5. Pigments of gliding bacteria. R as in Fig 6.

The chemical relationship between carotenoids from gliding bacteria (Fig. 5) and blue-green algae (33,34,75) is interesting. Blue-green algae are now reclassified as Cyanobacteria (Division I of the procaryotes) (27). Monocyclic carotenoid rhamnosides of myxoxanthophyll type are characteristic components. The carotenoid pattern may here offer support for a presumed phylogenetic relationship.

Before leaving the bacteria the possible significance of a minor variation in the dehydrogenation of phytoene to lycopene should be commented on. In photosynthetic bacteria the route via 7,8,11,12-tetrahydrolycopene (unsymmetrical ξ-carotene) may operate exclusively, in the C_{50}-carotenoid producer Flavobacterium dehydrogenans both unsymmetrical and symmetrical ξ-carotene have been detected and in higher organisms symmetrical ξ-carotene is common (76).

In addition to the C_{30} and C_{50} series of carotenoids encountered exclusively amongst bacteria and the unique carotenoids associated with photosynthetic bacteria, the missing ability of bacteria to synthesize acetylenic, allenic, epoxidic and nor-carotenoids should be noted. The latter statement also holds for yeasts and other fungi to be considered next.

Yeasts

Yeasts are not a taxonomic entity, but are unicellular fungi of particular subdivisions (77). Carotenoids are produced by species of the genera Sporobolymyces, Rhodotorula and Cryptococcus (42). Monocyclic carotenoids such as β,ψ-carotene, torulene and the carboxylic acid torularhodin are characteristic components. Presence of phytoene, phytofluene, ξ-carotene, neurosporene, β-zeacarotene and β,β-carotene has been demonstrated under particular conditions. Also the 17'-hydroxy and 17'-oxo intermediates expected for torularhodin formation have been isolated. Plectaniaxanthin and 2'-hydroxy-1,2-dihydrotorulene have been isolated from Cryptococcus laurentii (78), and 2-hydroxyplectaniaxanthin has been obtained from Rhodotorula aurantiaca (79,80).

To a plausible biosynthetic scheme given by Simpson (42) has been added the more recently described carotenoids from yeasts (Fig. 6), which all fall within a rather simple structural and biosynthetic pattern with dominance of monocyclic carotenoids.

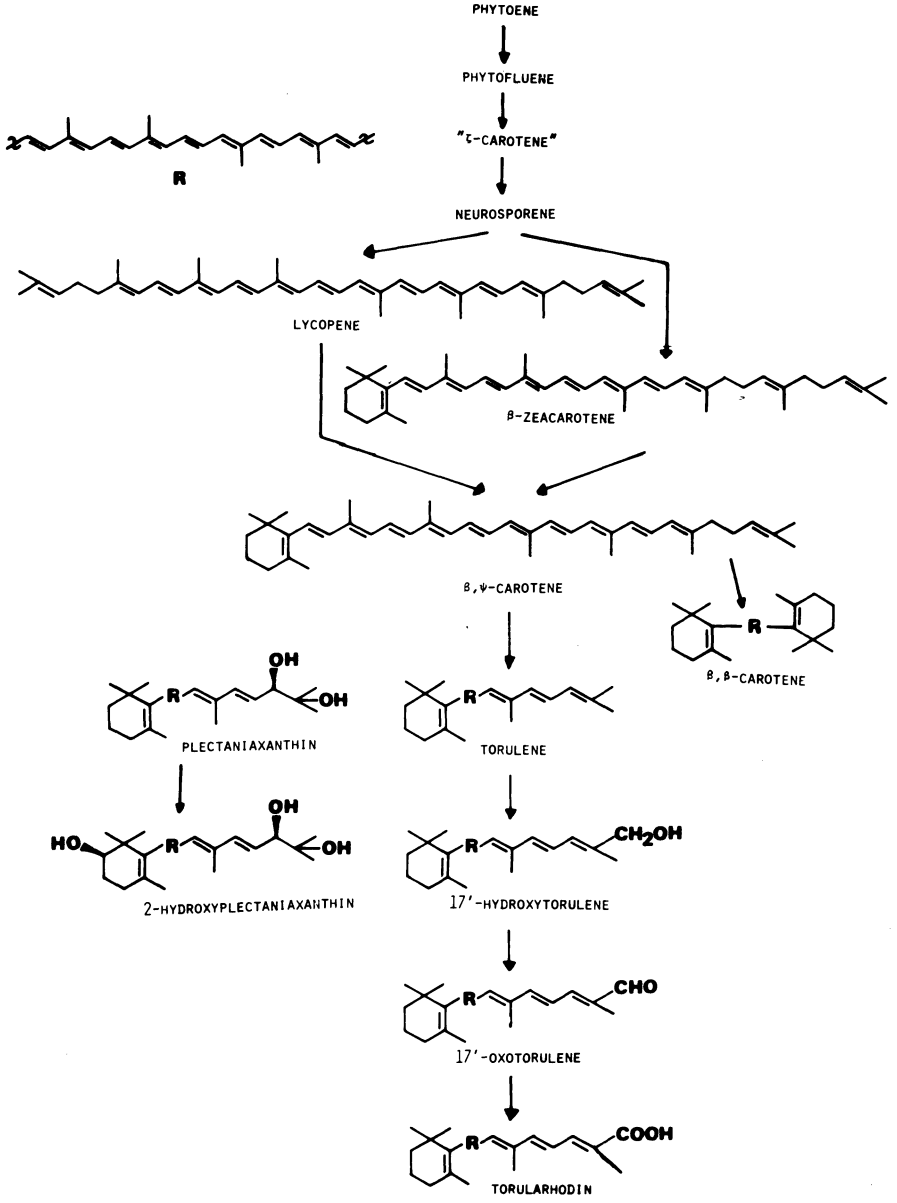


Fig. 6. Carotenoids from yeasts.

Other fungi

Early work has been reviewed by Goodwin (30), and carotenoids as taxonomic characters in fungi have been discussed (81). A detailed chemosystematic study of Discomycetes (a class of the division Ascomycotina) has been carried out by Arpin (82).

Various aliphatic, monocyclic and bicyclic carotenoids are encountered including representatives like neurosporaxanthin (83), 2'-dehydroplectaniaxanthin (84) and phillipsiaxanthin (85). Esterified xanthophylls are found: torularhodin methyl ester (86), esterified (higher fatty acids) aleuriaxanthin (87,88), plectaniaxanthin (80,84) and phillipsiaxanthin derivatives (84,85). β,γ-Carotene (89,90) is noteworthy. Other common carotenoids comprise β,ψ-carotene, torulene and β,β-carotene. The carotenoids encountered in fungi could be accounted for by the biosynthetic pathways roughly depicted in Fig. 7. Biosynthetic evidence for the formation of torularhodin has been reported (91,92).

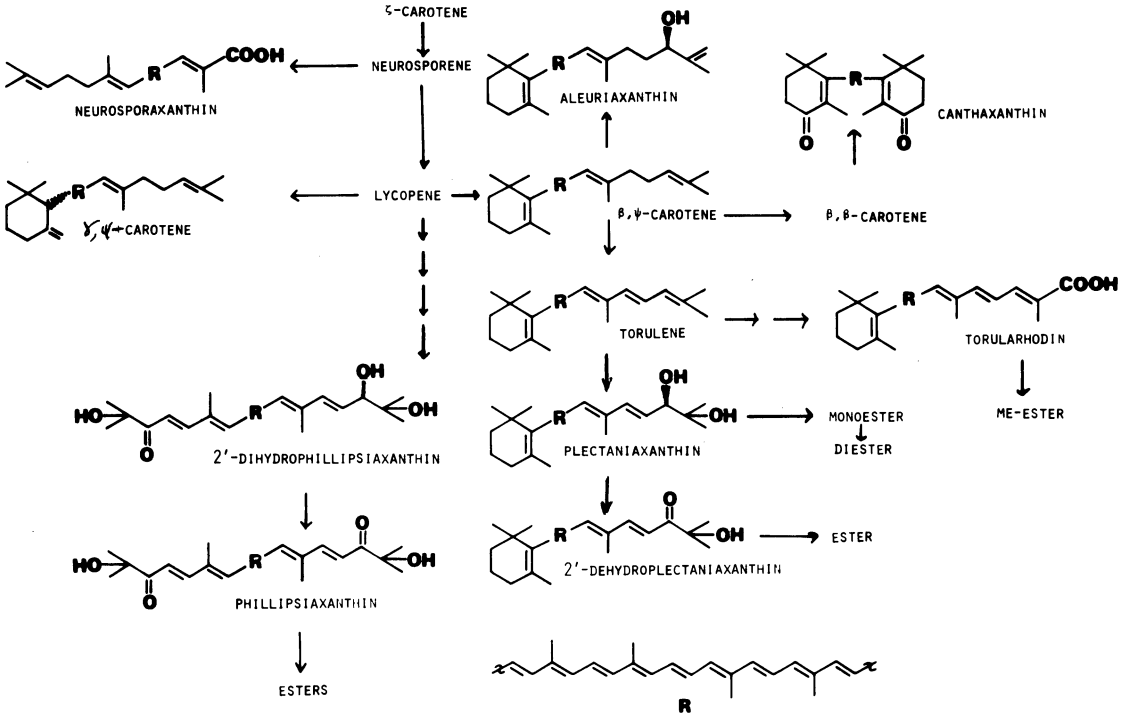


Fig. 7. Carotenoids of other fungi.

Algae

Let us now proceed to a consideration of carotenoids in algae. For the existing classification of algae (see 28) colour has been a major criterium (red, green, brown algae etc.). It is obvious that detailed structures of the compounds causing these colours offer a closer characterization. A detailed treatment of the carotenoids encountered in each algal class has recently been given elsewhere (75).

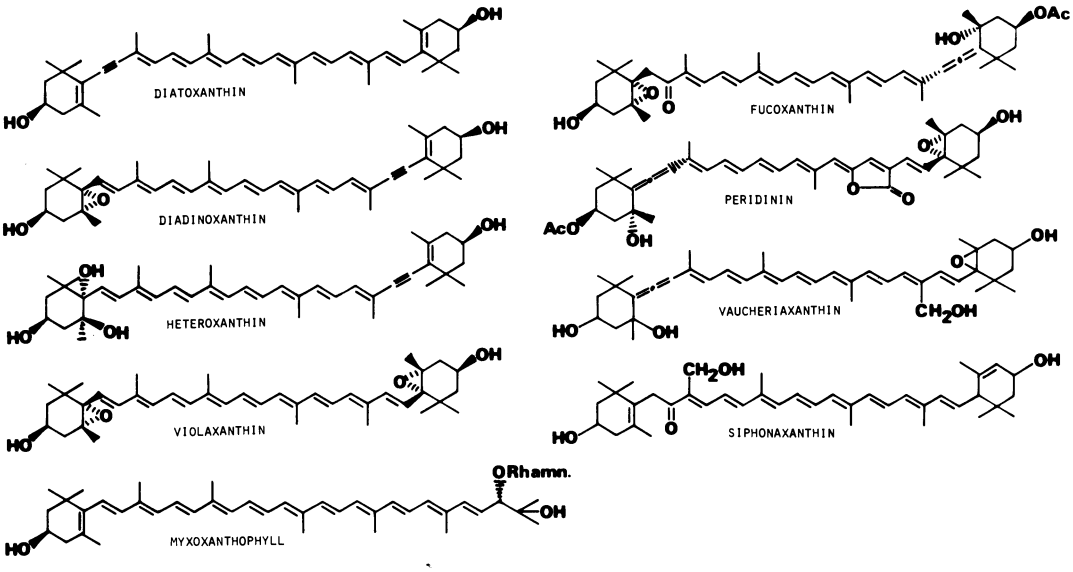
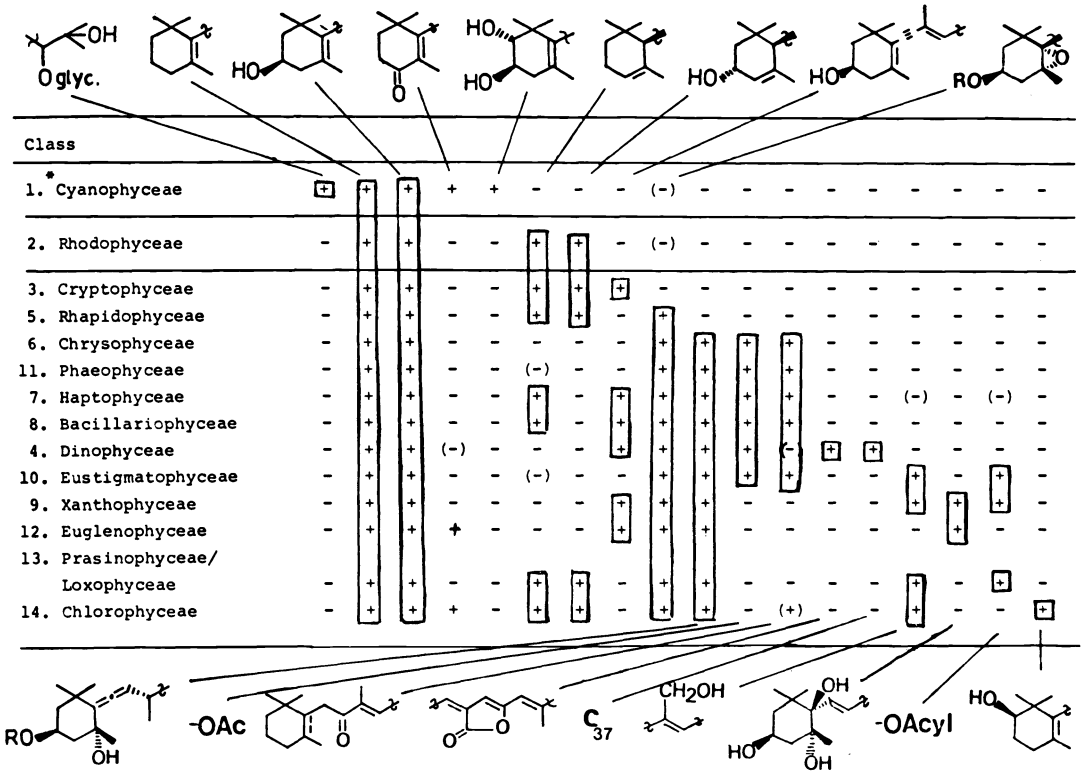


Fig. 8. Examples of characteristic algal carotenoids.

In Fig. 8 the structures of typical algal carotenoids are exemplified with diatoxanthin, diadinoxanthin, fucoxanthin, peridinin, heteroxanthin, vaucheriaxanthin (=19'-hydroxy-neoxanthin), siphonaxanthin, violaxanthin and myxoxanthophyll (80), demonstrating acetylenic, allenic, epoxy, acetoxy, 19-hydroxy, 8-keto, 5,6-glycol and glycosidic structural elements. Peridinin with a C_{37} carbon skeleton is noteworthy.
Table 2 (75) gives the various algal classes according to Christensen (28) and the structural features of the carotenoids encountered in each class.

TABLE 2. Structural features of the carotenoids encountered in the algal classes.



Monocyclic carotenoid rhamnosides of myxoxanthophyll type are peculiar to blue-green algae. Bicyclic xanthophylls are found in all algal classes and are of no chemosystematic value. 4-Keto-carotenoids encountered in blue-green algae, are minor constituents in Euglenophyceae (eye-spot pigments) and green algae grown under nitrogen starvation. ε-Rings and triple bonds are structural features restricted to the carotenoids of particular classes. Carotenoid epoxides are usually not encountered in the three most primitive algal classes. Exceptions are found within the subclass Florideophyceae of the red algae (35). The diepoxide violaxanthin is common in many green algae. Allenic carotenoids, 8-keto-carotenoids like fucoxanthin and siphonaxanthin, carotenol acetates and higher fatty acid esters are found as indicated. Butenolide formation and the C_3-expulsion probably involved in the biosynthesis of peridinin is a unique property of dinoflagellates. Oxidation of in-chain methyl groups to 19-ol is, however, effected also by other classes. 5,6-Glycol formation so far seems to be restricted to Xanthophyceae and Euglenophyceae. Finally 2-hydroxy-β-type carotenoids are found amongst Chlorophyceae.
To reach the final products in algal carotenoid synthesis a minimum number of some twenty step reactions are required from an aliphatic C_{40} precursor. Probable sequences, based on general knowledge of carotenogenesis and stereochemical evidence may be formulated (75,93). In Table 2 the structural features of the carotenoids are arranged not arbitrarily, but in an order which reflects the sequences of postulated step reactions, as far as it is possible to transfer a branched scheme to a linear system. The order of the algal classes has been changed somewhat in the center of Table 2 to obtain a syste-

matic change in the carotenoid pattern. The numbers to the left refer to the order in which the classes were treated by Christensen (28).
It is obvious that the structural diversity and distribution of algal carotenoids fall into a nice pattern useful for chemosystematics.
Some examples will now be given to illustrate this further:
On the basis of ultrastructure differences Hibberd and Leedale (94) transferred a number of species from the Xanthophyceae to the new class Eustigmatophyceae. This is nicely parallelled in carotenoid composition: acetylenic carotenoids being absent from Eustigmatophyceae (39,40).
Several previous Chrysophyceae spp. have been transferred to the Haptophyceae, now renamed Prymnesiophyceae (95), on morphological grounds (96). Recent studies on the carotenoids of Haptophyceae (38) and true Chrysophyceae spp. (97) indeed demonstrate a difference in carotenoid synthesis, since no acetylenic carotenoids were found in the true chrysophytes.
A challenge to the taxonomists is the following. The main carotenoid of dinoflagellates so far has been either peridinin or fucoxanthin, see Fig. 8. The recently studied dinoflagellate Gyrodinium cf. estuariale (98) contained as major carotenoid 19'-hexanoyloxyfucoxanthin (99), previously only encountered in Emeliana (Coccolithus) huxleyi (100,101) of the class Prymnesiophyceae ex Haptophyceae.
Turning from dinoflagellates to red algae, fucoxanthin has occasionally been isolated from red algae in quantities incompatible with diatom contamination. However, fucoxanthin was not present in cultured material of various species in which fucoxanthin was found in the "wild type" (35,102). Recently we have isolated peridinin as the main carotenoid from various Australian red algae and its origin may be disputed (103). Attempts to find halogenated carotenoids in red algae, known to contain halogenated terpenoids, have so far failed (103).
Although there are several uncertain points, consideration of the distribution pattern of individual carotenoids (and chlorophylls) in algae no doubt will gain increasing importance for the classification of the microscopic representatives.

Higher plants
Proceeding now to higher plants, epoxidic and allenic xanthophylls, but no acetylenic carotenoids are encountered. Green leaves are conservative in their carotenoid composition, Fig. 9. β,β-Carotene, β,ε-carotene, lutein, zeaxanthin, violaxanthin and neoxanthin are typical. Yellow flowers contain frequently complex mixtures of epoxidic carotenoids with insufficient variation for chemosystematic value. Cases like Eschscholtzia californica with eschscholtzxanthin (104,105) or Adonis annua with astaxanthin (106) are rather exceptions.

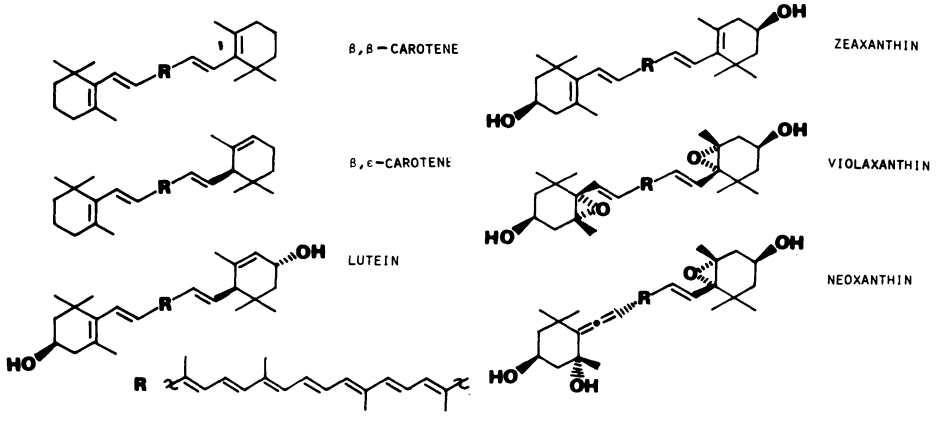


Fig. 9. Carotenoids of green leaves.

Various seeds, fruits and berries contain specific carotenoids, Fig. 10. Mentioned are capsanthin, capsorubin, bixin, crocetin, azafrin, other apocarotenoids, lycoxanthin, gazaniaxanthin, rubixanthin, celaxanthin, rhodoxanthin, ternstroemiaxanthin and 1,2-epoxides (29,107).

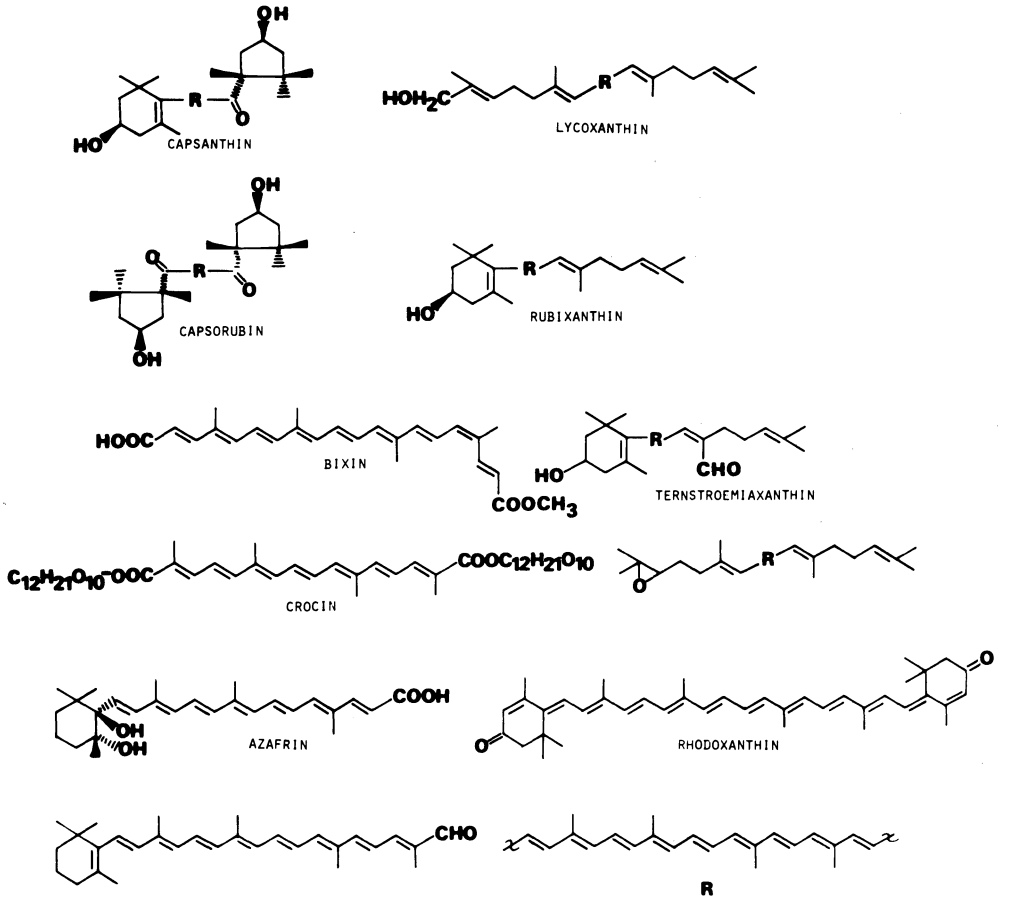


Fig. 10. Examples of carotenoids from seeds, fruits and berries.

Although useful for characterization at species level, carotenoids do not appear to be an important criterium for classification of higher plants.

Animals

Animals are presumably not able to carry out de novo carotenoid synthesis. Presence of carotenoids usually reflects symbiosis, resorption or metabolic capacity. In Fig. 11 are given examples of carotenoids not yet encountered in primary producers: 7,8,7',8'-tetradehydroastaxanthin, bound in Asterias rubens as a blue protein complex asteriarubin (108), actinioerythrin, known to have 3S,3'S-configuration (109), mytiloxanthin and isomytiloxanthin, trikentriorhodin, 7,8-didehydroisorenieratene, pectenolone and idoxanthin (29), all isolated from marine invertebrates. Added should also be the fish carotenoid tunaxanthin (110), an ε,ε-carotene-3,3'-diol stereochemically different from the chiriquixanthins from a frog (111). Astaxanthin is common in marine invertebrates. Recently we have demonstrated that the presumed astaxanthin-$CaCO_3$ complex from calciferous corals is a new carotenoprotein alloporin (112). Astaxanthin has, however, also been isolated from algae (36) and flowers (106).

Although of less direct value as chemosystematic markers than for the primary carotenoid producers, carotenoids in animals may demonstrate food chain relationships and metabolic capacity. The recent isolations of 2-hydroxy and 2-oxo bicyclic carotenoids (112,113), (3S,3'S)-astaxanthin (114) and philosamiaxanthin from insects and tunaxanthin (109) from fishes are interesting cases.

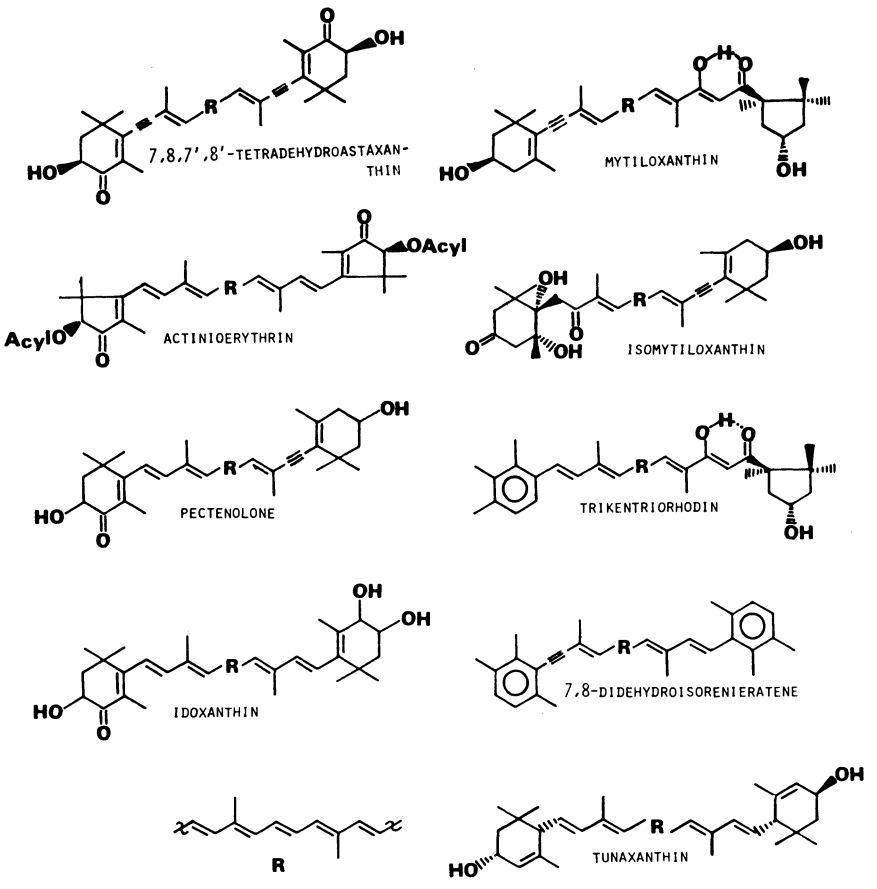


Fig. 11. Examples of metabolic carotenoids.

CONCLUSION

Table 3 gives the characteristic structural features of the carotenoids found in the main types of organisms. Systematic changes correlating in principle well with phylogenetic trees with hypothetic preflagellates succeeded by bacteria, blue-green and red algae, other algae, yeasts and fungi and higher plants, are found. Particularly at lower levels mere inspection of chemical details and biosynthetic pathways of carotenoids provides in most cases useful chemosystematic information.

TABLE 3. Characteristic structural features of the carotenoids in the main types of organisms.

de novo synthesis	Type of organism	Diapo C_{30}	C_{40}	C_{50}	Apo	Nor	Tert. -OMe	Glyc.	Aromatic	Acetylene	Epoxide	Allene	Me-oxid.	Acetate	Other esters
	Bacteria Photosynthetic		+		(+)		+	+	+				+		
	Non-photosynthetic	+	+	+				+	+						
	Yeasts		+		+								+		
	Fungi		+		+								+		+
	Algae		+			+		+		+	+	+	+	+	+
	Higher Plants		+		+						+	+	+		+
	Animals		+		+	+			+	+	+	+	+	+	+

If a third dimension like functional aspects could be added, such as protection by specific carotenoids against photodynamic damage (116,117), protective colours by true carotenoproteins or carotenoid-glycolipoprotein complexes (118,119), carotenoid-chlorophyll-protein light harvesting complexes (120), role in reproduction (117,121) etc. could be more closely defined, terms like biochemical systematics or biofunctional systematics are also expected to be appropriate in carotenoid context.
However, carotenoids of course only represent one piece in a great puzzle. In the future computerized treatment of all chemical constituents, biosynthetic pathways and functional aspects of secondary metabolites, together with the other criteria discussed in the introduction of this lecture, should contribute to the demonstration of phenetic and phylogenetic relationships. Numerical taxonomy (122) based on several characters is still a young science.

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