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CAROTENOIDS AS ADDITIONAL TAXONOMIC CHARACTERS IN FUNGI: A REVIEW

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The aim of this review is twofold: first to discuss some of the pitfalls of carotenoid identification in fungi and secondly to observe to what extent carotenoids may be useful as taxonomic characters. The results suggest that a number of carotenoids have been wrongly identified and that with newer techniques available, new carotenoids will almost certainly be identified which may help taxonomists. In a number of cases carotenoids are very good taxonomic markers.

It is well known that in higher plant tissue, carotenoids are closely tied up with chlorophyll and are useful during photosynthesis. The carotenoids of leaves seem to vary little, consisting mainly of β-carotene and lutein and of some of their derivatives. However, flowers and fruits tend to have certain carotenoids specific to themselves, e.g. rose hips (rubixanthin, I), woody nightshade (lycoxanthin, II and lycophyll, III) and *Gazania rigens* flowers (gazaniaxanthin, IV) (Valadon, Sellens & Mummery, 1975). In the algae, carotenoids are good taxonomic characters. Valadon (1966) attempted to show that this may be the case in fungi and concluded that much more information was required before any definite conclusions could be reached. Chemical taxonomy has interested a great number of workers in the last 10 years or so and with more recent studies available it may be possible to take another look as to whether or not carotenoids may be good taxonomic markers in fungi.

From the earlier works of Zopf (1890) and Kohl (1902) it became known that a number of fungi contained carotenoids but it was left to later workers to identify these pigments more accurately. As newer methods of identification were used it became obvious that a number of the earlier works which identified carotenoids, mainly by absorption spectra and by co-chromatography, were wrong. There are a number of carotenoids with similar absorption spectra and some of these may be similarly adsorbed on impregnated paper or on various columns and may not be separable using these techniques, yet they may be different compounds. Spirilloxanthin (V) restricted to certain photosynthetic purple bacteria had been identified in *Lycogala epidendron*, *Aleuria aurantia* (Lederer, 1938) and *Neurospora crassa* (Haxo, 1949) but was later shown to be absent from these organisms (Liaaen-Jensen, 1965).

Liaaen-Jensen (1965) suggests that 3,4-dehydrolycopene (VI) had been previously mistaken for spirilloxanthin (V) in *Neurospora crassa* and this is more readily understandable in view of the fact that this carotenoid was rapidly formed in *N. crassa* from more saturated carotenoid precursors (Grob, 1958). On the other hand, Haxo (1949) had obtained his pigment as a major component (27% total carotenoid) whereas 3,4-dehydrolycopene was only a minor component (2%) of Liaaen-Jensen's (1965) cultures. If it were the same compound in the two cases then some mutation or a different strain was the cause of such observed differences, since these compounds with similar absorption spectra were found in different amounts in the two strains used.

Another example of mistaken identity was given by Valadon & Mummery (1975*b*) who showed that what was identified as neurosporene (VII) by Turian (1960) and Fiasson (1968) in *Cantharellus infundibuliformis* might in fact be an expoxycarotenoid (VIII). This compound, which was found to make up 72% total carotenoids (Fiasson, 1968), was also the major component of both Turian's (1960) and Valadon & Mummery's (1975*b*) samples. It had the same adsorption spectrum as neurosporene (415, 440 and 470 nm in hexane) but on the modified conc. HCl-ether test of Jungalwala & Cama (1962) it was converted to its furanoid oxide (IX) (absorption spectrum 380, 400 and 420 nm in hexane). This compound cannot be neurosporene and was shown to be an expoxycarotenoid.

A further point worth making is that pathways of carotenoid biosynthesis in fungi seem to have

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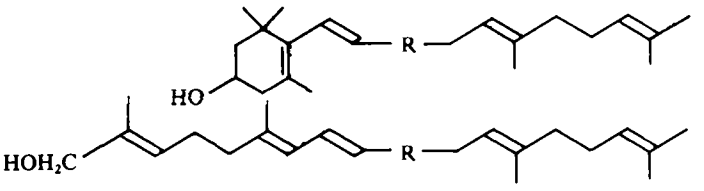
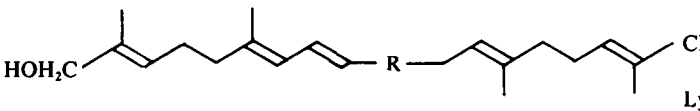
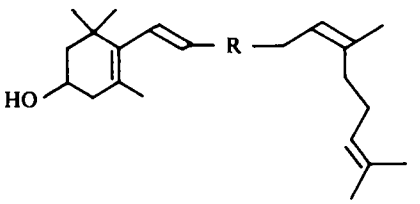


Rubixanthin
(I)

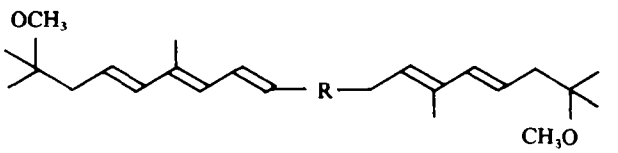
Lycoxanthin
(II)



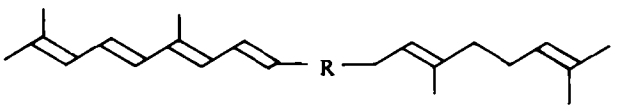
Lycophyll
(III)



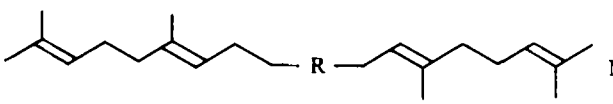
Gazaniaxanthin
(IV)



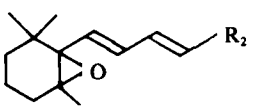
Spirilloxanthin
(V)



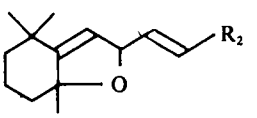
3,4-Dehydrolycopene
(VI)



Neurosporene
(VII)



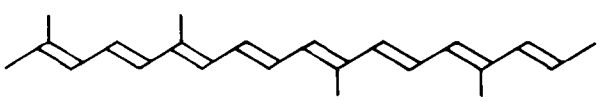
5,6-Epoxy-carotene
(VIII)



5,8-Furanoid epoxide
(IX)

Key to the chemical formulae (pages 2, 4, 7):

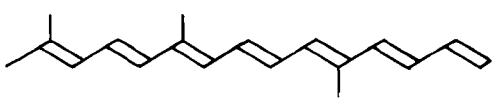
R=



R_2 = unknown in this particular case

R_3 and R_4 = fatty acids

R_5 =



R_6 = Acyl radical

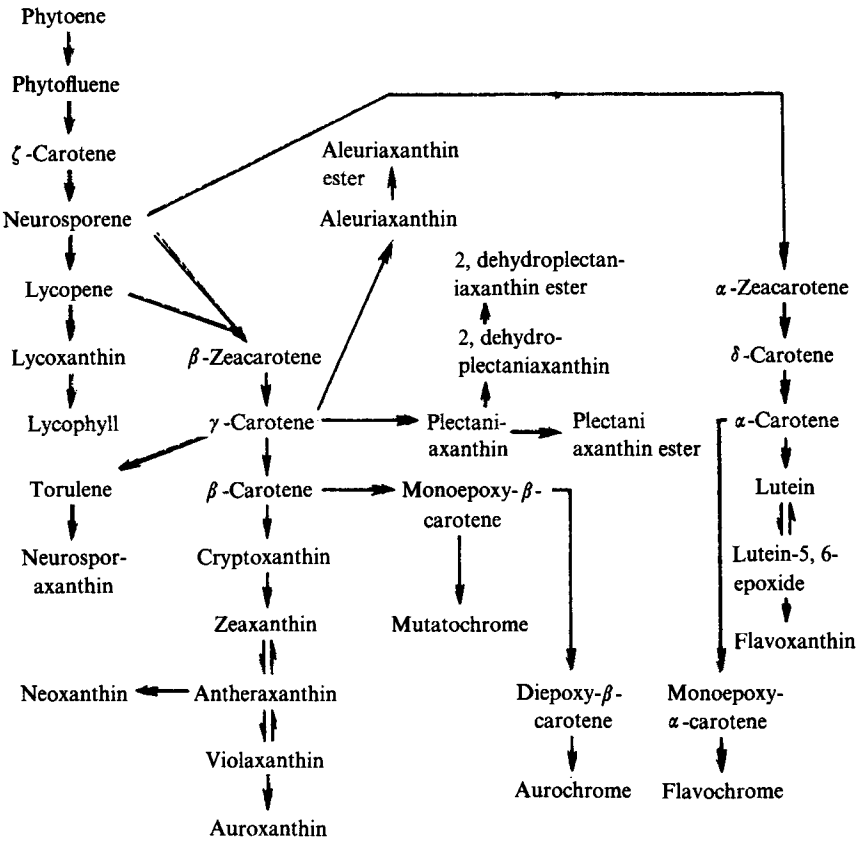
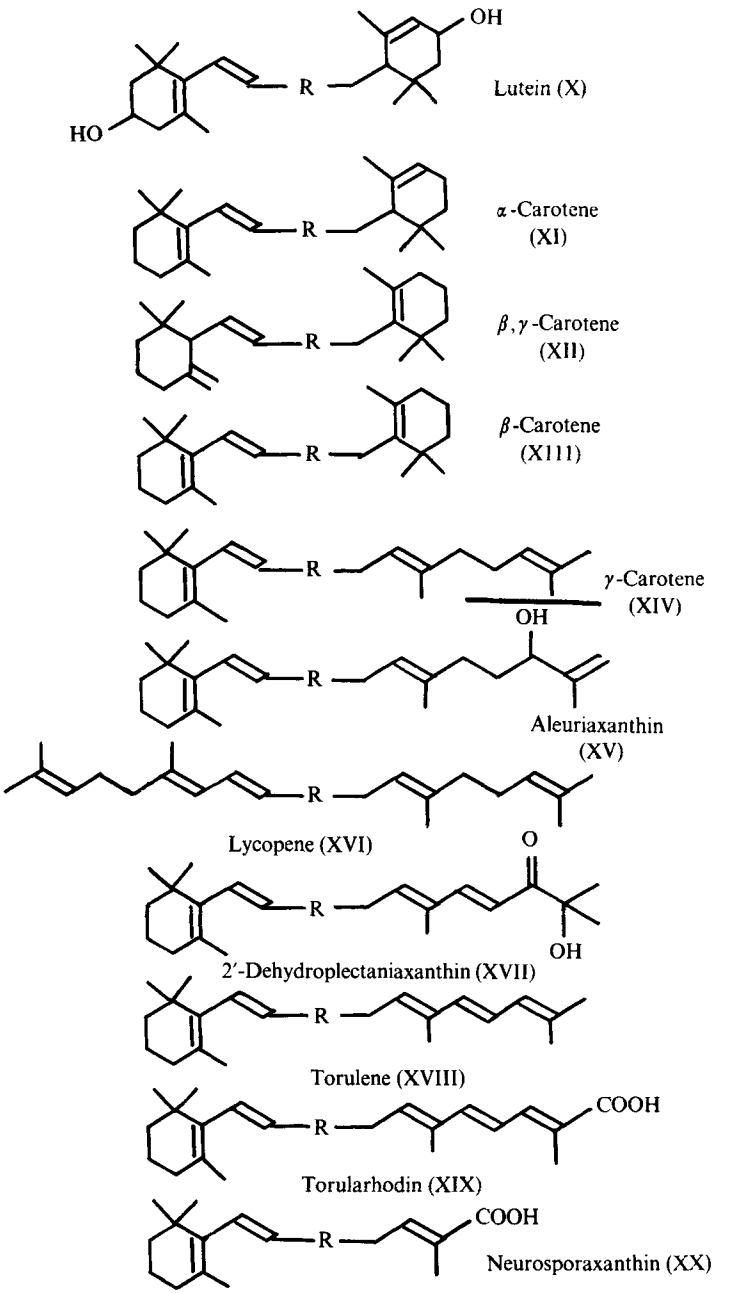


Fig. 1. Suggested pathways of the synthesis of α-, β- and γ-carotenes and of their derivatives (Fiasson, 1968; Valadon & Mummery, 1975*a*)

evolved along a different line to that of photosynthetic tissues (Fig. 1). No lutein (X) has been found in fungi although in a few cases small amounts of α-carotene (XI) have been reported. The identification of α-carotene is not reliable because the pigment with absorption spectrum 420, 444 and 475 in hexane and weakly absorbed on column chromatography is not always α-carotene.

Arpin *et al.* (1971) showed that a carotene in *Caloscypha fulgens* was, β, γ-carotene (XII), a new pigment with a terminal methylene group, which could easily be taken for α-carotene. Fiasson (1968) using the newer methods of identification could not identify α-carotene in certain fungi which were earlier reported to contain it.

These examples suggest that most fungi will have to be re-investigated using modern techniques. Certainly new carotenoids will have to be characterized before they become helpful to the taxonomist. Another aspect which needs considering is the fact that different investigators not only extend the range of carotenoids found by previous workers but also fail to find those originally identified. The following example will illustrate this. Lederer (1938) obtained a dried specimen of *Aleuria aurantia* (Vienna, Austria) and identified β-(XIII), γ-carotene (XIV) and possibly ribixanthin (I) (458, 490 nm in petroleum ether). Although Valadon (1964*a*) was able to obtain eight) bands after MgO-Celite (1:1) v/v column chromatography, he positively identified only the same three carotenoids in specimens of *A. aurantia* obtained in Windsor Great Park, England. Co-chromatography with rubixanthin (I) from rose hips suggested that a fourth pigment identified as rubixanthin? by the latter worker was indeed rubixanthin. Liaaen-Jensen (1965) obtained two lots of *A. aurantia* from two locations in Norway and found only slight differences between their carotenoids (Table 1) and identified a new fungal carotenoid, aleuriaxanthin ester (XV), in both. The first batch had almost equal quantities of β-(XIII) and γ-carotene (XIV), minute amounts of lycopene (XVI) and 3,4-dehydrolycopene (VI) and 25% aleuriaxanthin (XV) ester. The second batch on the other hand had twice the amount of



γ-carotene as β-carotene and no 3,4-dehydrolycopene, otherwise it was identical to the first batch. She found no ribixanthin although 1 % of her total carotenoids was not identified (Table 1). Valadon & Mummery (1968) obtained a number of specimens of this fungus from Englefield Green, Surrey, England, and were able to separate twelve bands, of which they identified the following eight carotenoids: α-, β-, γ-carotene, neurosporene, lycopene, 3,4-dehydrolycopene, rubixanthin and aleuriaxanthin ester. Later on that year Arpin (1968) obtained 200 g dry weight of specimens of *A. aurantia* from Theys (Isère), France, and characterized a new pigment, dehydro-2′-plectaniaxanthin (XVII) and its ester, as well as β-, γ-carotenes, torulene (XVIII) and aleuriaxanthin

Table 1. *Carotenoids found in* Aleuria aurantia *(Pers. ex Hook.) Fuckel by various authors. The results are expressed as percentage total carotenoids*

Carotenoids	A. *aurantia* from Vienna, Austria (Lederer, 1938)	*A. aurantia* from Windsor Great Park, England (Valadon, 1964*a*)	*A. aurantia* from Jonsvannet, Norway (Liaaen-Jensen, 1965)	*A. aurantia* from Withelmsyr, Norway (Liaaen-Jensen, 1965)	*A. aurantia* from Englefield Green, England (Valadon & Mummery, 1968)	*A. aurantia* from Theys, France (Arpin, 1968)
α-Carotene	–	–	–	–	0·3	–
β-Carotene	+	40·0	34·0	26·0	43·9	38·0
γ-Carotene	+	27·6	39·0	50·0	15·5	36·0
Rubixanthin	?	28·7	–	–	9·9	–
Neurosporene	–	–	–	–	4·3	–
Aleuriaxanthin ester	–	–	25·0	22·0	21·5	20·0
Lycopene	–	–	0·2	1·0	1·7	–
3,4-Dehydrolycopene	–	–	1·0	–	0·8	–
Torulene	–	–	–	–	–	+
Dehydro-2′ plectaniaxanthin	–	–	–	–	–	0·5
Dehydro-2′ plectaniaxanthin ester	–	–	–	–	–	4·0
Unidentified	+	3·7	1·0	1·0	2·1	1·5
Total carotenoids (mg/g dry wt)		0·82	1·65	1·50	0·68	1·50

(XV) ester. He was able to identify torulene in traces even though this had not previously been characterized in *A. aurantia* by other workers. This is an example whereby carotenoids appearing in traces could be positively identified because large amounts of material were available for analysis. He did not obtain rubixanthin or 3,4-dehydrolycopene and suggested that these two compounds could have been wrongly identified aleuriaxanthin ester (or an isomer) and/or the ester of dehydro-2′-plectaniaxanthin. He may have been right but in the four cases where aleuriaxanthin ester has been identified it made up between 20 and 25 % total carotenoids. Valadon & Mummery (1968) and Liaaen-Jensen (1965) obtained 3,4-dehydrolycopene as well as aleuriaxanthin ester, so these two are different compounds. What was identified as rubixanthin (Valadon & Mummery, 1968) may have been another ester of aleuriaxanthin having the same chromatographic properties as rubixanthin but not the aleuriaxanthin ester of Liaaen-Jensen (1965) and Arpin (1968), which was also characterized by them (Valadon & Mummery, 1968). On the other hand, *A. aurantia* (Liaaen-Jensen, 1965; Arpin, 1968) has been shown to have two carotenoids (XV, XVII) with hydrolylation on positions 1′ and 2′ respectively and it is therefore not unexpected that in some strains the hydroxylation may be at the 3 position as in rubixanthin (I).

This brings us to the question of different strains and mutation in fungi. In the Fungi Imperfecti it has not been easy to classify the asporogenous yeasts but Lodder & Kreger-van Rij (1952) proposed two subdivisions of the family Cryptococcaceae, one lacking carotenoids (Cryptococcoideae) and the other containing carotenoids (Rhodotoruloideae). However, Nakayama, Mackinney & Phaff (1954) found that whereas certain species of Rhodotorula contained predominantly torulene (XVIII) and/or torularhodin (XIX), other species of this genus and also all species of *Cryptococcus* studied contained mostly β-carotene with minor amounts of γ-carotene and lycopene. Temperature was a very important factor contributing to the degree of pigmentation and they concluded that carotenoids were so variable in these fungi that a separation of the two genera on the basis of manifest carotenoids was vague and arbitrary. Further, Villoutreix (1960) produced a number of mutants of *R. mucilaginosa* through u.v.-irradiation; some of these did not contain carotenoids although the wild type did. This was also shown for *R. glutinis* (Nusbaum-Cassuto, Villoutreix & Threlfall, 1965). Valadon & Heale (1965) demonstrated another aspect of fungal metabolism by producing coloured mutants by u.v.-irradiation of the colourless, wild-type *Verticillium albo-atrum*. Some of these mutants had large amounts of lycopene and/or of torulene and of neurosporaxanthin (XX), while the wild-type contained only the colourless C_{40} precursor, phytoene (Fig. 1). This shows that it is possible to produce carotenoid mutants artificially in fungi.

Arpin (1968) collected a number of coloured strains of *Sarcoscypha coccinea* and analysed their carotenoids content. The results (Table 2) showed

Table 2. *Carotenoids of different coloured strains of* Sarcoscypha coccinea (*Jacq. ex S. F. Gray*) *Lamb.* (=Plectania coccinea (*Scop. ex Fr.*) *Fuckel* (*Arpin*, 1968). *The results are expressed as percentage total carotenoids*

	Strains					
Carotenoids	(Normal) red–orange	Lot 1 orange	Lot 2 orange	Lot 3 orange	Lot 4 orange	Cream
β-Carotene	24·0	90·0	76·0	90·0	75·0	+
β-Zeacarotene	–	–	8·0	–	–	–
γ-Carotene	–	–	3·5	–	–	–
Torulene	–	10·0 ?	12·0	9·0	13·0	–
Plectaniaxanthin	3·0	–	–	–	–	–
Plectaniaxanthin (monoester)	6·0	–	–	–	–	–
Plectaniaxanthin (diester)	47·0	–	0·5 ?	–	–	–
Dehydro-2′plectaniaxanthin	20·0	–	–	–	–	–
Unidentified	–	–	–	1·0	12·0	–
Total carotenoids (mg/100 g)	160·0	70·0	60·4	79·1	75·2	trace

?, Not identified with certainty.

that there were differences between the cream, orange and red-orange strains of this fungus. There was very little carotene in the cream strain, between 60 and 80 mg/100 g dry weight in the four orange strains and usually about 160 mg/100 g in the normal red-orange strains (several strains were analysed but only one instance given in this case). Not only were there differences between total carotenoids but also among individual carotenoids. Plectaniaxanthin (XXI) and its derivatives (plectaniaxanthinmonoester XXII, diester XXIII and dehydro-2′-plectaniaxanthin XVII) were the dominant pigments in the red-orange wild-type, and β-carotene in the orange 'mutants'. Differences do therefore occur in nature and one must be very careful about generalization.

TAXONOMIC IMPLICATIONS

Cantino & Hyatt (1953) were probably the first authors to use carotenoids as one of their markers in suggesting evolutionary relationships between *Blastocladiella* and *Rhizophlyctis*. Mutants of *Blastocladiella* were obtained which possessed certain characteristics in common with both *Rhizophlyctis rosea* and *B. emersonii* and these results suggested a close relationship between these two genera. Moreover they found that whereas wild-type *Blastocladiella* possessed α-ketoglutarate oxidase activity and no detectable carotenoids, the two mutants of *Blastocladiella* and the wild-type *Rhizophlyctis rosea* possessed γ-carotene and no detectable α-ketoglutarate oxidase activity. The results they obtained, coupled with the carotenoid picture, offered direct and undisputable evidence for the origin, from a blastocladiaceous fungus, of an organism which closely resembled the chytridiaceous *R. rosea* and possibly the mode of origin in nature of *Rhizophlyctis* and *Blastocladiella* (Cantino & Hyatt, 1953).

It was not until Turian (1960) did some biochemical work on various fungi that the first suggestion was made of the possible use of carotenoids as a taxonomic marker. He showed that the orange-red *Guepinia helvelloides* did not contain carotenoids but quite likely anthraquinones; Lederer (1938) had earlier shown that *Tremella mesenterica* contained β-carotene. On this basis, Turian (1960) suggested that this is a further character which supports the separation of the tribes; Tremelleae (containing carotenoids) and Guepineae (containing anthraquinones) among the large family of Tremellaceae. The genus *Guepinia* is replaced by Martin (1944) by that of *Dacryopinax*, a member of the family Dacrymycetaceae which also includes *Dacrymyces*, and *Calocera* while *Tremella* is a member of Tremellaceae. There are therefore other marked differences between these two genera. However, it is members of the Dacrymycetaceae which on the whole contain carotenoids, e.g. *Dacrymyces*, *Calocera* and *Dacryopinax* and therefore carotenoids are not good taxonomic characters in this case. This shows that we cannot be too careful about deductions from data based on only two species.

Among the lower Ascomycetes a number of fungi have been found, the life cycles of which are relatively difficult to work out (Valadon, 1961). Species of *Protomyces* and of *Taphrina* macroscopically resemble one another, although *T. deformans* causes peach leaf curl and *P. inundatus* causes galls on *Apium nodiflorum*. In culture they look alike, bud in a yeast-like manner and are pigmented. A very simple way of differentiating

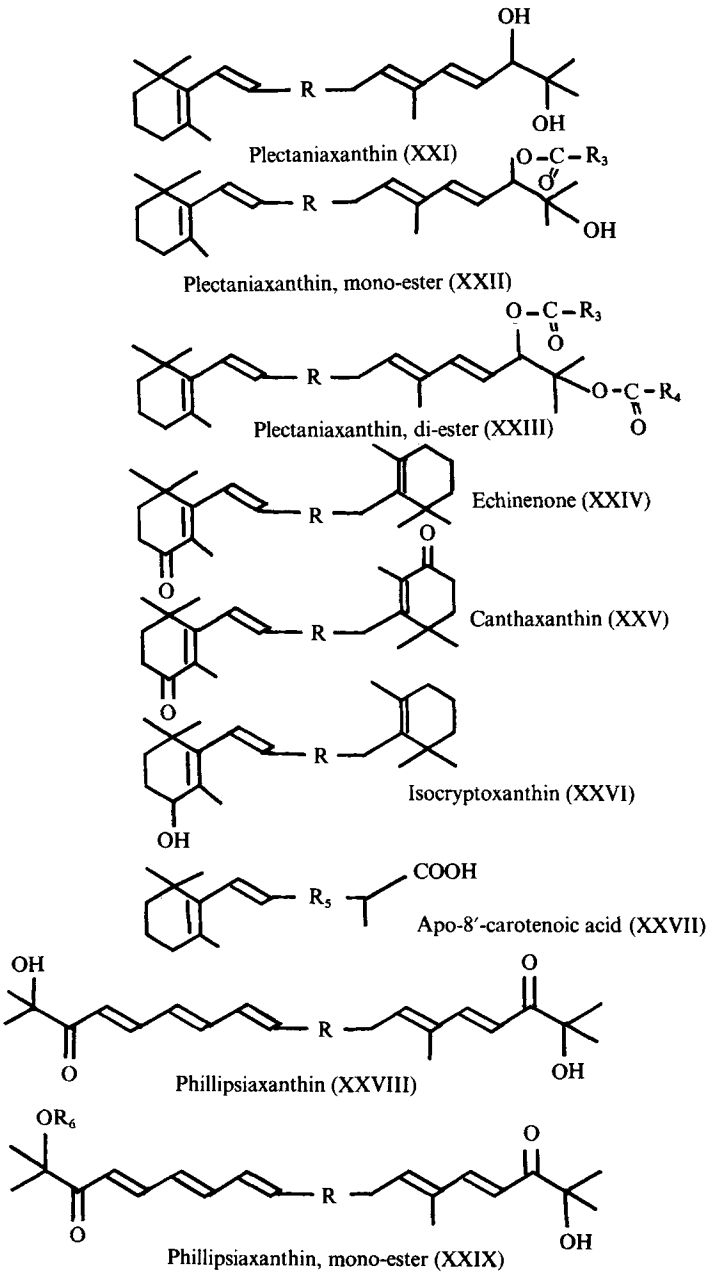


Table 3. *Carotenoids in three species of* Protomyces (*Valadon*, 1963, 1964*b*) *and their absence in two species of* Taphrina. *The results are expressed as percentage totals carotenoids*

Species	α-Carotene	γ-Carotene	β-Carotene	Lycopene	Total carotenoids (μg/g dry wt)
Protomyces inundatus Dangeard	5·0	8·0	87·0	+	7·4
P. inouyei P. Hennings	–	4·2	93·7	2·1	4·9
P. pachydermus Thuemen	–	1·2	95·8	3·0	5·8
Taphrina deformans (Berk.) Tul.	–	–	–	–	–
T. communis (Sadeb.) Giesenh.	–	–	–	–	–

Table 4. *Carotenoids of certain Phycomycetes*

Phycomycetes	Neuro-sporene	Lyco-pene	β-Zea-carotene	ζ-Caro-tene	γ-Caro-tene	β-Caro-tene	Refer-ences*
Chytridiales							
Rhizophlyctis rosea (de Bary & Woronin) Fischer	−	+	−	−	+++	−	1
Cladochytrium replicatum Karling	−	+	−	−	+++	−	2
Blastocladiales							
Blastocladiella spp. Matt.	−	−	−	−	+++	−	3
Allomyces arbuscula Butler	−	−	−	−	+++	−	4
A. macrogynus (Emerson) Emerson & Wilson	−	−	−	−	+++	−	4
A. moniliformis Coker & Braxton	−		−	−	+++	−	4
A. javanicus Emerson	−			+	+++	+	4, 5
Mucorales							
Blakeslea trispora Thaxt.	−	+	+	+	+	+++	6
Choenophora cucurbitarum (Berk. & Rav.) Thaxt.	−	−	−	−	−	+++	7
Mucor hiemalis Wehmer	+	+	+	−	+	+++	8
Phycomyces blakesleanus Burgeff	+	+	+	+	+	+++	9
P. nitens (Agardh.) Kunze ex Fr.	+	+	−	+	+	+++	10
Pilaira anomala (Ces.) Schroet.	−	−	−	−	+?	+++	11
Pilobus kleinii van Tiegh.	−	−	−	−	−	+++	12
Syzygites megalocarpus Ehrenberg ex Fr.	−	+	−	−	+	+++	13

+++ indicates the major carotene and + indicates presence.

* 1, Davies (1961); 2, Fuller & Tavares (1960); 3, Arpin (1968); 4, Emerson & Fox (1940); 5, Turian & Haxo (1954); 6, Anderson *et al.* (1958); 7, Barnett, Lilly & Krause (1956); 8, Herber (1974); 9, Goodwin (1952); 10, Goodwin & Griffiths (1952); 11, Fletcher (1969); 12, Fiasson (1968); 13, Wenger & Lilly (1966).

between these two genera in culture is by extracting the pigments. Whereas *P. inundatus*, *P. inouyei* and *P. pachydermus* contains carotenoids, *T. communis* and *T. deformans* do not (Table 3). Therefore one can easily distinguish between these two genera (Valadon, 1963, 1964*b*) although more information on other species of these two genera would be most welcome.

Another example where carotenoids have been used to differentiate between two genera is given by Arpin (1966). *Clitocybe venustissima* which was previously called *Hygrophoropsis* is a bright orange Agaric which may be confused with *Hygrophoropsis aurantiaca*, yet β- and γ-carotenes have been identified in the former with the complete absence of carotenoids in the latter. This, together with other morphological data, supports the placing of these two fungi in different genera.

A further case may be mentioned here. The Thraustochytriaceae was placed by Sparrow (1943) as a family of the Saprolegniales (Oomycetes) as they are chytrid-like organisms that reproduce by means of biflagellate zoospores. *Schizochytrium aggregatum* belongs to this family and was recently shown to have very different cell wall characteristics to those of typical Oomycetes (Darley, Porter & Fuller, 1973). These authors have therefore concluded that this family should be dissociated from the Oomycetes: carotenoid studies have strengthened their conclusions. Porter & Valadon (1975) have shown that *S. aggregatum* contains the following carotenoids: α-, β-carotenes, echinenone (XXIV), canthaxanthin (XXV) and possibly isocryptoxanthin (XXVI). Very few fungi are known to contain keto-carotenoids, like echinenone and canthaxanthin (Fiasson, 1968) and they certainly have not yet been identified in the Oomycetes so far studied. Ketocarotenoids are typical animal carotenoids and are also common among algae and these results are in agreement with the suggestion that 'there are similarities between the Thraustochytriaceae and the marine creepy-crawlie Labyrinthula' (Darley *et al.* 1973).

If one takes a closer look at the carotenoids identified in the Phycomycetes, one finds that members of the Chytridiales and Blastocladiales so far studied contain γ-carotene as their major pigment, whereas the Mucorales contain β-carotene as the major one (Table 4) so it is possible to separate these three Orders into two groups according to the main pigment present.

It is among the Discomycetes that a systematic attack was launched in an attempt to obtain as much information as possible about carotenoids and taxonomy. Arpin (1968) studied both the operculate and the inoperculate Discomycetes. First, a look at Dennis' (1960) taxonomic account of British Cup Fungi shows how difficult it is to

Table 5. *Carotenoids of operculate Discomycetes* (*Arpin*, 1968). *See Table 2 for details of* Sarcoscypha *spp. which may be part of the subfamily Sarcoscypheae*

Pezizales	Neurosporene	Lycopene	γ-Carotene	Torulene	β,γ-Carotene	Hydroxy-β,γ-Carotene	β-Carotene	Torularhodin-methylester	Aleuriaxanthin-ester	Plectaniaxanthin	Plectaniaxanthin-ester	Dehydro-2′-plect-aniaxanthin ester	Phillipsiaxanthin ester	P510-ester	Unidentified
Family Aleuriaceae *sensu* Arpin															
Group 1															
Sowerbyella unicolor (Gill.) Nannf.	−	−	+	+	+	+	+ + +	−	−	−	−	−	−	−	−
S. radiculata (Sowerby ex Fr.) Nannf.	−	−	+	−	+	+	+ + +	−	−	−	−	−	−	−	+
Caloscypha fulgens (Pers.) Boud.	+	+	+	+	+	−	+ + +	−	−	−	−	−	−	−	+
Anthracobia melaloma (Alb. & Schw. ex Fr.) Boud.	+	−	+	?	−	−	+ + +	−	−	−	−	−	−	−	−
Pulvinula constellatio (Berk. & Br.) Boud.	−	−	+	−	−	−	+ + +	−	−	−	+	−	−	−	+
Group 2															
Leucoscypha rutilans (Fr.) Dennis & Rifai.	−	+	+	−	−	−	+ + +	−	−	+	−	−	−	−	+
Aleuria rhenana Fuckel	−	−	+	+	−	−	+ + +	−	−	−	−	−	−	−	+
Melastiza chateri (Smith) Boud.	−	−	+	−	−	−	+	−	+ + +	−	−	+	−	−	+
M. greletii Le Gal	−	−	+	−	−	−	+	−	+ + +	−	−	+	−	−	−
Octospora calichroa (Boud.)	−	−	−	−	−	−	+	−	−	−	+ + + ?	−	−	−	−
O. leucoloma Hedwig ex S. F. Gray	−	−	+	−	−	−	+	−	−	+ + +	−	−	−	−	−
Group 3															
Coprobia granulata (Bull. ex Fr.) Boud.	−	−	+ + +	−	−	−	+	−	−	−	−	−	−	−	+
Cheilymenia theleboloides (Alb. & Schw.) Boud.	−	−	+ + +	−	−	−	−	−	−	−	−	−	−	−	+
C. crucipila (Cooke & Phillips) Le Gal	−	−	+ + + ?	−	−	−	−	−	−	−	−	−	−	−	−
Scutellinia setosa (Nees ex Fr.) Kuntze	−	−	+	−	−	−	+ + +	−	−	−	−	−	−	−	−
S. umbrarum (Fr.) Lambotte	+	−	+ + +	+	−	−	−	−	−	−	−	−	−	−	+
S. arenosa (Vel.) Le Gal	−	−	+ + +	+	−	−	−	−	−	−	−	−	−	−	+
S. superba (Vel.) Le Gal	−	−	+ + +	+	−	−	−	−	−	−	−	−	−	−	+
S. scutella var. *cervorum* (Vel.) Le Gal	−	−	+ + +	+	−	−	−	−	−	−	−	−	−	−	−
S. ampullaceae (Limm.) Kuntze	−	−	+ + +	+	−	−	−	−	−	−	−	−	−	−	+
S. trechispora (Berk. & Br.) Lambotte	−	−	+ + +	+	−	−	−	−	−	−	−	−	−	−	−
Geopyxis carbonaria (Alb. & Schw.) Sacc.	−	−	−	+ + +	−	−	−	−	−	−	−	−	−	−	+ + +
G. majalis (Fr.) Boud.	−	−	+	+	−	−	+	−	−	−	−	−	−	−	+ + +
Family Sarcoscyphaceae															
Subfamily Sarcoscypheae															
Pyronema omphalodes (Bell. ex. Fr.) Fuckel	−	−	−	−	−	−	−	+ + + ?	−	−	−	−	−	−	+
*P. confluens** Tul. (= P. omphalodes)	−	−	+	+	−	−	+	−	−	−	−	−	−	−	+
Pithya vulgaris Fuckel	−	−	−	−	−	−	+ + +	−	−	+	+	−	−	−	−
Cookenia sulcipes (Berk) Kuntze	?	+	−	−	−	−	−	+	−	−	−	−	+ + +	+	+
C. tricholoma (Mont.) Kuntze	−	−	−	−	−	−	−	−	−	−	−	−	+ + +	+ + +	+
Phillipsia carminea (Pat.) Le Gal	−	+	−	−	−	−	−	−	−	−	−	+	+ + +	+	−
P. subpurpurea Berk. & Br.	−	+	−	−	−	−	−	+	−	−	−	−	+ + +	−	+
P. carnicolor Le Gal	−	−	−	−	−	−	−	−	−	−	−	−	+ + +	−	−

* Also contains celaxanthin (= 3-hydroxytorulene). + + +, Major carotenoid; +, carotenoid present.

differentiate between the various families and the tribes therein. The Pezizales which comprise all species with operculate asci are divided into six families, only two of which contain carotenoids, namely the Humariaceae and the Sarcoscyphaceae. The Humariaceae is divided into three tribes: Lachneae, Ciliarieae and Aleurieae. 'In Boudier's system tribe Lachneae comprises seven genera represented in Britain but it must be admitted that several of these are separated on rather ill-defined grounds'. Also 'five genera are conventionally recognized in the tribe Ciliareae but the distinction between *Scutellinia*, *Cheilymenia* and *Neottiella* is not altogether satisfactory. *Melastiza* may have affinities with *Aleuria* in the other tribe Aleurieae'. The third tribe is Aleurieae 'a residual assemblage of genera, defined largely by negative characters; the absence of a blue ascus tip in iodine, the absence of clearly-differentiated hairs and the absence of broad asci protruding conspicuously above the hymenial level at maturity'. It is evident that it is not easy to differentiate not only between the families but also between the genera. Moreover other taxonomists (Le Gal, 1963; Rifai, 1968) are rather divided in what constitutes tribes and what constitutes families. Arpin (1968) having analysed a great number of fungi from these groups for their carotenoids content, and finding no other distinctive criterion, felt that on pigmentation characteristics he could group the tribes Ciliarieae and Aleurieae into the family Aleuriaceae Arpin and the tribes Lachneae and Otideae into the Otideaceae of Eckblad (1968). He then divided the Aleuriaceae into three groups: (1) the first contained β-carotene as the major pigment, e.g. *Sowerbyella*, *Caliscypha*; (2) the second β-carotene and γ-carotene and its derivatives (XV, XXI), e.g. *Aleuria*, *Melastiza*; (3) the third γ-carotene, e.g. *Coprobia*, *Scutellinia* (Table 5).

The family Sarcoscyphaceae is not a homogeneous group and the tribe Sarcoscypheae containing large amounts of xanthophylls is not related in any way to the other tribe Urnuleae which contains hydrophilic pigments (Arpin, 1968). Some of the genera of Sarcoscypheae contain large amounts of a novel pigment phillipsiaxanthin (XXVIII) and its esters. The monoester is represented as (XXIX) where R_6 is an acyl radical. The presence of this unusual pigment could be important taxonomically. Although it would simplify matters greatly to use certain carotenoids as main taxonomic markers, as was attempted by Arpin (1968), it is not always possible to do so and other characteristics must also be taken into account. The latest classification of the Discomycetes incorporates both fungal morphology and some of Arpin's (1968) work. Korf (1973) names two suborders: Sarcoscyphineae and Pezizineae; the former is divided into two families Sarcosomataceae and Sarcoscyphaceae each divided into two tribes. One of the characters he uses to separate the Sarcosomataceae from the Sarcoscyphaceae is that apothecia of the former are dark-coloured with melanin-like pigments and excipulum devoid of carotenoids; but such pigments rarely occur in the paraphyses whilst apothecia of the latter are bright-coloured, usually with carotenoids in the hymenium and often also in the excipulum. Korf (1973) divides the Sarcoscyphaceae into two tribes depending on whether all asci within each apothecium mature simultaneously (Boedijnopezizeae) or asci of various ages within a single apothecium mature successively (Sarcoscypheae). This separates the genus Cookeina (Boedijnopezizea) from Phillipsia (Sarcoscypheae) which were both shown to have the rare carotenoid phillipsiaxanthin as their major carotenoid and which could be placed together in the Sarcoscypheae (Table 5).

Korf (1973) divides the suborder Pezizineae into five families. The presence and absence of carotenoids are used in two of the families: Ascobolaceae and Pyronemataceae. The family Ascobolaceae comprises two tribes, Ascoboleae and Iodophaneae; the latter has five genera, two of which may be separated by using the characteristics of the apothecia, supplemented by the presence and absence of carotenoids. Iodophanus has lenticular to convex apothecia, almost always with carotenoids, whilst Trecotheus has subconical to cylindrical or turbinate apothecia and is devoid of carotenoids.

The family Pyronemataceae on the other hand is divided into five subfamilies: Ascodesmidoideae, Ascophanoideae, Otideoideae, Scutellinioideae and Pyronematoideae, the first three lacking carotenoids altogether. Scutellinioideae and Otideoideae are easily separable in that the ascospores may be guttulate in both, yet carotenoids are present in the former. The subfamily Scutellinioideae is divided into three tribes namely Sowerbyelleae, Aleurieae and Scutellinieae; the first contains β-carotene as the major pigment and carotene P.444 (unknown elsewhere in the Pezizales), the fungus discolouring greenish or red to brownish on bruising, the second β-carotene or both β- and γ-carotenes as major pigments but not discolouring on bruising, whilst the third contains γ-carotene as the main pigment. These divisions are broadly speaking the same as those of Arpin (1968) who calls them Groups 1, 2 and 3 respectively (Table 5). This is yet another instance where carotenoids have helped to reinforce morphological characters in fungal taxonomy.

Arpin (1968) next looked at pigmentation from

Table 6. *Carotenoids of certain yeasts: various species of* Cryptococcus, Rhodotorula, Sporidiobolus *and* Sporobolomyces

	Neuro-sporene	Lyco-pene	γ-Carotene	Toru-lene	Toru-larhodin	β-Carotene	Refer-ences†
Cryptococcus flavus (Saito) Phaff & Fell (=*R. flava* (Saito) Lodder)	−	+	+	−	−	+++	1
C. laurentii (Kuff.) Skinner var. *flavescens* (Saito) Lodder & Kreger-van Rij (=*R. aurea* (Saito) Lodder)	−	+	+	−	−	+++	1
C. laurentii (Kuff.) Skinner var. *flavescens* (Saito) Lodder & Kreger-van Rij (=*R. peneaus* Phaff, Mrak & Williams)	−	+	+	−	−	+++	1
C. laurentii (Kuff.) Skinner var. *laurentii*	−	+	+	−	−	+++	1
C. luteolus (Saito) Skinner	−	+	+	−	−	+++	1
*Rhodotorula aurantiaca** (Saito) Lodder	−	−	+	−	−	+	2, 3
R. glutinis (Fr.) Harrison	+	−	+	+	+++	+	1, 3
R. gracilis Rennerfelt (=*R. glutinis* (Fres.) Harrison)	−	−	+	+++	−	+	2
R. infirmominiata (Okunuki) Hasegawa & Banno (=*C. infirmominiatus* (Okunuki) Phaff & Fell)	−	−	+	+++	−	+	1
R. minuta (Saito) Harrison	−	−	+	+++	+	+	1, 3
R. mucilaginosa (Jörg.) Harrison (=*R. rubra* (Demme) Lodder)	+	+	+	+	+++	+	4, 5
R. palida Lodder	−	−	+	+++	−	+	1
R. rubra (Demme) Lodder	+	−	+	+	+++	+	1
R. sanniei (Cif. & Red.) Lodder (=*R. rubra* (Demme) Lodder)	−	?	+	+	+++	+	2
Sporidiobolus johnsonii Nyland	−	−	+	+	+++	+	6
Sporobolomyces roseus Kluyver & Van Niel	−	−	−	+	+++	+	2, 7
S. pararoseus Olson & Hammer (=*S. ruber* Yamazaki & Fujii)	−	−	?	+	+++	+	2
S. salmonicolor (Fisher & Brebeck) Kluyver & Van Niel	−	−	−	+	+++	+	2, 7

* The major pigment 'P475' has not yet been identified (Fiasson, 1968).

+++, Major carotenoid; +, carotenoid present.

† 1, Nakayama *et al.* (1954); 2, Fiasson (1968); 3, Peterson *et al.* (1958); 4, Bonaly & Villoutreix (1965); 5, Villoutreix (1960); 6, Fiasson (1967); 7, Lederer (1938).

an evolutionary viewpoint. Harborne (1967) has argued that if different compounds can be placed in sequence on a biosynthetic pathway,

$$A \rightarrow B \rightarrow C \rightarrow D \rightarrow E,$$

then a plant/organism making only A has fewer enzymes at its disposal than one containing E, i.e. E is more advanced from an evolutionary point of view. Since the inoperculate Discomycetes only contained β-carotene as their major carotenoid (Arpin, 1968), they are not considered as advanced as the operculate Discomycetes which have γ-carotene and its oxygenated derivatives as major pigments (Fig. 1). The same argument presumably applies to the Phycomyces since the Mucorales with β-carotene as major pigment will be more evolved than either the Chyrtidiales or the Blastocladiales with γ-carotene only as their major carotenoid.

Carotenoids have been implicated as taxonomic markers among yeasts. Lodder & Kreger-van Rij (1952) separate the Cryptococcaceae (no carotenoids) from the Rhodotorulaceae (presence of carotenoids) but later studies (Nakayama *et al.* 1954; Petersen *et al.* 1958) showed that a number of Cryptococci did contain carotenoids. Lodder (1970), following Phaff & Spencer's (1969) suggestion, separates the two in that *Cryptococcus* assimilates inositol as a single carbon source and *Rhodotorula* does not. Phaff & Fell (1970) found that this division removed all starch positive species from Rhodotorula and this appeared to correlate well with the chemical composition of the capsular polysaccharides of the two genera. Moreover, they could not accept the division of *Rhodotorula* suggested by Hasegawa, Banno & Yamauchi (1960) into *Flavotorula* (colonies reddish to pale yellow or pale orange) and *Rubrotorula* (colonies red to orange) because of the great variability in types and concentration of carotenoids in different strains and with changes in environmental conditions. However, if conditions

Table 7. *Carotenoids of various species of* Cantharellus *and* Craterellus. *The results are expressed as percentage total carotenoids*

Species	Neuro-sporene	Lyco-pene	P.444	γ-Caro-tene	β-Caro-tene	Echi-nen-one	Cantha-xan-thin	Others	Total carote-noids (μg/g dry wt)	Refer-ences†
Subgenus: Cantharellus										
Cantharellus cinnabarinus Schw.	−	−	−	−	+	+	+++	−	30·0	1
Ca. friesii Quél.	+	−	−	+	+++	++	+	+	15·7	1
Ca. cibarius Fr. var. *pallidifolius* Smith	−	+	+	+	+++	−	−	+	3·0	1
Ca. cibarius Fr. (cream spores) Pet.	−	?	+	+	+++	−	−	+	4·0	1
Ca. cibarius Fr. (yellow spores) Pet.	−	+	−	+	+++	−	−	+	10·0	1
Ca. minor Pk.	−	−	+	−	+++	−	−	−	57·0	1
Subgenus: Phaeocantharellus										
Ca. lutescens Fr.	+++	+++	−	−	−	−	−	+	*	1
Ca. cf *lutescens* Fr. (No. 3540)	+	+++	−	−	−	−	−	+	3·3	1
Ca. cf *lutescens* Fr. (No. 3543)	++	+++	−	−	−	−	−	+	0·6	1
Ca. infundibuliformis Fr.	+++	+	−	−	−	−	−	+	20·0	1
Ca. infundibuliformis Fr.	+++	+	−	−	−	−	−	+	10·0	2
Ca. infundibuliformis Fr.	−	+	−	−	+	−	−	++×	457·5	3
Ca. ianthinoxanthus (R. Maire) Kuhner	26	74	−	−	−	−	−	−	18·0	4
Craterellus cornucopioides Pers per Fr.	+++	++	−	−	−	−	−	+	0·6	1
Cr. fallax Smith	−	+	−	+	+++	−	−	+	0·7	1
Cr. odoratus (Schw.) Fr.	−	+	−	+	+++	−	−	+	1·3	1
Cr. cinereus (Fr.) Quél.	−	−	−	−	−	−	−	−	0	1, 2

+++, Major carotenoid.
++, Second major pigment (between 30–45 %).
++×, The major carotenoid is an expoxycarotenoid but NOT neurosporene.
*, No amount given.
† 1, Fiasson *et al.* (1970); 2, Turian (1960); 3, Valadon & Mummery (1975*b*); 4, Fiasson (1973).

are kept constant, carotenoid studies of these two genera correlate well with the other above-mentioned parameters (assimilation of inositol and starch positiveness among *Cryptococcus*) in that *Cryptococcus* contains β-carotene as its major carotenoid whilst Rhodotorula have either torulene or its carboxlyated derivative torularhodin (Table 6). Since torularhodin is further along the biosynthetic pathway than β-carotene, then *Rhodotorula* will be considered more advanced than *Cryptococcus*.

Two other genera of yeasts that may be closely related are Sporidiobolus and Sporobolomyces, both characterized by the presence of ballistospores and indistinguishable in the young state although the latter lack clamp connexions (Phaff, 1970). Fiasson (1967) has supported the close relationship between these two genera since *Sporidiobolus johnsonii*, *Sporobolomyces roseus* and *S. salmonicolor* have the same carotenoids, torularhodin being the major pigment with smaller amounts of torulene and β-carotene (Table 6).

Corner's (1966) treatise on Cantharelloid fungi gives one a first-hand insight into the problems of classifying this group of fungi. He divides *Cantharellus* into three subgenera: Cantharellus, Phaeocantharellus and Cantharellotus, and separates these from the genus *Craterellus* on the form of their fruit bodies, the nature of the hymenial folds or ridges and in the short-celled hyphae without clamps in the latter. '*Craterellus* has been much confused with *Cantharellus*' he quotes. These points are considered further after investigations on the carotenoids of a number of species in this group (Fiasson, 1973; Fiasson, Petersen, Bouchez & Arpin, 1970) (Table 7). It can be observed from these results that the subgenus Cantharellus contains β-carotene as the major pigment and the subgenus Phaeocantharellus either neurosporene or lycopene or both. On this

basis they were in agreement with Corner's (1966) scheme. *C. ianthinoxanthus* had been placed in the subgenus Cantharellus but was later found to be more closely related morphologically to the Phaeocantharellus group (Fiasson, 1973). The carotenoids too agreed closely with this since it was found to contain neurosporene and lycopene. Fiasson *et al.* (1970) conclude as *Craterellus* is a heterogeneous group it is not surprising that some species, e.g. *Cr. cornucopioides* have the same carotenoid composition as the subgenus Phaeocantharellus and others, e.g. *C. fallax* and *C. odoratus* the same as the other subgenus Cantharellus. It is obviously time to dispense with the genus *Craterellus* as such, since the genera containing carotenoids would fit in nicely as Cantharellus. On the other hand one might be expected to keep the genus *Craterellus* for those where no carotenoids are present, e.g. *C. cinereus*. The point already raised which must be remembered in this context is the true identity of the pigment called neurosporene here. It would be interesting to reanalyse these various species to ascertain if *Ca.* cf *lutescens* No. 3540 or any of the other Phaeocantharellus contained neurosporene or the epoxycarotenoid (Valadon & Mummery, 1975*b*) which was found in specimens of *Ca. infundibuliformis*. Fiasson *et al.* (1970) have suggested that the genus *Cantharellus* may be easily divided into the subgenus Cantharellus (containing the bicyclic carotenoid β-carotene and its ketonic derivatives) and the subgenus Phaeocantharellus (containing aliphatic carotenoids like neurosporene and lycopene). The accumulation of large amounts of neurosporene in nature is unusual and Turian (1960) suggested that *C. infundibuliformis* might contain a natural inhibitor because only in the presence of inhibitors like diphenylamine does neurosporene accumulate in large amounts in certain micro-organisms. However, if what was identified as large amounts of neurosporene in *Ca. lutescens*, *Ca. infundibuliformis* and in *Cratellerus cornucopoides* is found to be an expoxycarotenoid (Valadon & Mummery, 1975*b*) then Fiasson *et al.*'s (1970) argument will still be valid provided one replaces aliphatic carotenoids by epoxycarotenoids for the subgenus Phaeocantharellus.

Another taxonomic point among the Basidiomycetes was raised by Valadon & Mummery (1969) who extracted laetiproxanthin from *Laetiporus sulphureus*. This fungus was known as *Polyporus sulphureus* but was found to be morphologically different from the genus *Polyporus* and was placed in the new genus *Laetiporus* (Pegler, 1966). Although laetiporxanthin has not yet been characterized it may well be β-apo-8-carotenoic acid (XXVII) already synthesized (Isler *et al.* 1959) but which has not yet been found in nature. The presence of a carotenoid in this fungus and its absence in *Polyporus grammocephalus*, *P. luzonenis*, *P. rubidis* and *P. zonalis* (Bose, 1941) shows yet another instance where a biochemical approach supports evidence obtained on morphological grounds.

CONCLUSIONS

Although carotenoids are not universally present in fungi they may still be good taxonomic markers. Other characters being equal, presence and absence of these pigments would help to separate two genera. When a systematic approach was instituted on various Discomycetes, Arpin (1968) used the presence of carotenoids to form a new family Aleuriaceae; the genera involved were found to have a good affinity with one another. Again among the asporogenous yeasts there has been a certain amount of controversy as to what constituted a genus. With the help of certain other characters supplemented by carotenoid studies one should be able to separate species of *Rhodotorula* containing torulene or torularhodin from those of *Cryptococcus* which contain β-carotene as their major pigment.

It is possible to use carotenoids in differentiating the subgenera *Cantharellus* and *Phaeocantharellus* in that the former contain β-carotene as their major pigment whilst the latter either 'neurosporene' or lycopene or both (Fiasson *et al.* 1970). Furthermore it is suggested that since differences are not always clear cut among the Cantharelloid fungi (Corner, 1966), species of *Craterellus* which contain carotenoids may be absorbed by Cantharellus and only those without carotenoids will keep their same generic status. This would be a helpful marker for taxonomists because various species among these two genera are constantly alternating between being called *Craterellus* and *Cantharellus*.

Finally, although carotenoids may help the taxonomist a great deal, it would be worth while re-investigating the carotenoids of a number of species using new techniques to ascertain the correct structure of these compounds. This was not always possible in the past. New compounds will most certainly be identified which may help the taxonomists further.

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