2. The decreased synthesis of β-carotene and the increased synthesis of the phytofluene group which occurs in the presence of diphenylamine is directly proportional to the concentration of the inhibitor.

3. No compound of similar structure or oxidation-reduction potential has been found which has the same effect on carotenogenesis as diphenylamine. Phenol is also inactive. 2-Naphthylamine stimulates β-carotene synthesis.

4. Ferrous sulphate does not counteract the action of diphenylamine. Either AMP (muscle adenylic acid) or riboflavin returns the synthesis of β-carotene to normal in diphenylamine cultures, but neither lowers the synthesis of the more reduced polyenes to normal. Yeast adenylic acid is without effect.

5. AMP alone has no effect on the growth, lipid production or carotene production by *Phycomyces*.

6. In growing cultures, riboflavin, up to a concentration of 1/20000, stimulates growth, lipogenesis and carotenogenesis; at higher concentrations inhibition of the three processes occurs. In older cultures the same effect is observed with growth and carotenogenesis, but lipogenesis is stimulated at all concentrations of riboflavin tested.

7. Mats cultured in the presence of diphenylamine and containing large amounts of the phytofluene group do not convert these into β-carotene, when washed and transferred to non-diphenylamine media.

REFERENCES

Burkholder, P. R., McVeigh, I. & Moyer, D. (1944). *J. Bact.* **48**, 385.

Chargaff, E. & Lederer, E. (1935). *Ann. Inst. Pasteur*, **54**, 383.

Garton, G. A., Goodwin, T. W. & Lijinsky, W. (1951). *Biochem. J.* **48**, 154.

Goodwin, T. W. (1952). *Biochem. J.* **50**, 550.

Goodwin, T. W., Griffiths, L. A., Jamikorn, M. & Willmer, J. A. (1952). *2nd Int. Congr. Biochem. Abstr.* p. 210.

Goodwin, T. W. & Jamikorn, M. (1952*a*). Unpublished observations.

Goodwin, T. W. & Jamikorn, M. (1952*b*). *Nature, Lond.*, **170**, 104.

Goodwin, T. W. & Lijinsky, W. (1952). *Biochem. J.* **50**, 268.

Goodwin, T. W. & Willmer, J. S. (1952). *Biochem. J.* **51**, 213.

Kharasch, M. S., Conway, E. A. & Bloom, W. (1936). *J. Bact.* **32**, 533.

Lederer, E. (1938). *Bull. Soc. Chim. biol., Paris*, **20**, 611.

Nord, F. F., Fiore, J. V., Kreitman, G. & Weiss, S. (1949). *Arch. Biochem.* **23**, 480.

Turian, G. (1950). *Helv. chim. acta*, **33**, 1988.

Turian, G. (1951). *Helv. chim. acta*, **34**, 1060.

Van Niel, C. B. & Smith, J. H. C. (1935). *Arch. Mikrobiol.* **6**, 219.

Studies in Carotenogenesis

8. THE CAROTENOIDS PRESENT IN THE BASIDIOMYCETE *DACROMYCES STILLATUS*

By T. W. GOODWIN

Department of Biochemistry, The University of Liverpool

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Although *Phycomyces blakesleeanus* is in many ways an excellent organism for the study of carotenogenesis (Garton, Goodwin & Lijinsky, 1951), it has certain disadvantages, chief of which is that it cannot be grown in shake cultures. Other carotenogenic fungi which have been examined recently, e.g. *Rhodotorula* spp. (Lederer, 1938; Bonner, Sandoval, Tang & Zechmeister, 1946) and *Neurospora* spp. (Haxo, 1949), are more suitable from this point of view, but themselves suffer from two disadvantages; (*a*) some of the constituent carotenoids are unstable and (*b*) it is extremely difficult to extract quantitatively the pigments, thus precluding the accurate routine examination of a large number of cultures.

A survey of the old observations of Zopf (1890) and Kohl (1902) concerning the occurrence of lipochromes in fungi suggested that *Dacromyces stillatus* might fulfil the present requirements. An investigation into the suitability of this organism was therefore begun. It soon became apparent, however, that it was not going to fulfil our requirements, mainly owing to its inability to grow quickly and reproducibly on fully defined media. A pre-

liminary study on the identification of the carotenoids present provided information of some interest; this was, therefore continued, and the results are now reported.

EXPERIMENTAL

Culture of organisms. *Dacromyces stillatus* was obtained from Baarn and cultured at 28° on a medium containing: glucose, 2·50; L-asparagine, 0·20; $MgSO_4.7H_2O$, 0·05; KH_2PO_4, 0·15; yeast extract, 0·20%. *Rhodotorula rubra* was cultivated under the same conditions and *Phycomyces blakesleeanus* under the conditions previously described (Garton *et al.* 1951).

Preparation of the unsaponifiable extracts. The mycelia of *Dacromyces* and *Rhodotorula* were ground up with anhydrous Na_2SO_4 as previously described for *Phycomyces* (Garton *et al.* 1951) and extracted with acetone, which was a much more effective extracting solvent than ether. The lipids of *Phycomyces* were extracted in the usual manner. The unsaponifiable fractions of all three extracts were obtained using the method of Goodwin & Morton (1946). The unsaponifiable fraction of *Dacromyces* was dissolved in a small amount of light petroleum (b.p. 40–60°) and the carotenoids separated as follows:

Separation 1. This was carried out on a mixture of 4 parts activated alumina (Spence, grade 'O') and 1 part alumina deactivated with methanol (Goodwin, 1952*a*), using light petroleum containing 15% (v/v) ether as developer. The resulting chromatogram is described in Table 1.

Table 1. *The separation of* Dacromyces *carotenoids on a* 4:1 *mixture of activated and deactivated alumina, using light petroleum containing* 20% *(v/v) ether as developer*

(The zones are numbered in order of increasing adsorptive power.)

Zone no.	Description	Absorption spectrum maxima in light petroleum (mμ.)
1	Colourless; slight green fluorescence	—
2	Yellow; trace of green fluorescence	446, 473
3	Broad orange-red	450, 476
4	Trace of yellow-orange	425, 450
5	Narrow brown-orange	459, 489
6	Pinkish orange	451, 480, 513

Separation 2. Fraction 6 (Table 1) was rechromatographed on weakened alumina using light petroleum containing 10% (v/v) ether as developer. Four zones separated, as described in Table 2.

Examination of the fractions. After further chromatographic purification the following fractions were provisionally identified: (1) phytofluene, (2) α-carotene, (3) β-carotene, (4) ζ-carotene (Table 1), (6*B*) torulene, (6*C*) cryptoxanthin and (6*D*) zeaxanthin (Table 2). Confirmatory tests were carried out using authentic samples of the pigment concerned. The carotenes required for comparison were obtained from *Phycomyces blakesleeanus* (Goodwin, 1952*a*), cryptoxanthin and zeaxanthin from the berries of *Lonicera japonica* (Goodwin, 1952*b*) and torulene from a culture of *Rhodotorula rubra* (Lederer, 1938).

Quantitative experiments. In order to determine the relative amounts of the pigments present in *Dacromyces*, the fractions obtained as described above in two experiments were dissolved in known volumes of light petroleum and the extinctions of these measured at the wavelength of maximal absorption of the pigment concerned. Using the $E^{1\%}_{1cm.}$ values previously reported (Goodwin, 1952*a*, *b*) and assuming that for torulene at 490 mμ. to be 2500, the amounts of each pigment present could be calculated.

Table 2. *The separation of fraction* 6 (*Table* 1) *into its constituents on a column consisting of deactivated alumina, using light petroleum containing* 10% *(v/v) ether as developer*

Zone no.	Description	Absorption spectrum maxima in light petroleum (mμ.)
6*A*	Trace of lemon-yellow	—
6*B*	Pink	484, 515
6*C*	Orange	450, 474
6*D*	Orange-red strongly held	451, 476

RESULTS

(*a*) *Carotenoids present*

Fractions 1–4 were identified as phytofluene, α-carotene, β-carotene and ζ-carotene, respectively. Using the spectroscopic and chromatographic criteria previously described in detail (Goodwin, 1952*a*, *b*), they were found to be indistinguishable from authentic specimens of the suspected polyenes.

Fraction 5, which occurred in very small traces, was in all probability γ-carotene, but this could not be completely confirmed.

The first runnings of fraction 1 contained a little material with an absorption spectrum exhibiting maxima at 285 and 298 mμ. in light petroleum; this was in all probability phytoene (Porter & Lincoln, 1950) but, again, so little was present that complete identification was not possible.

Fractions 6*C* and 6*E* were identified as cryptoxanthin and zeaxanthin respectively (Goodwin, 1952*b*).

Fraction 6*B* was compared with an authentic specimen of torulene. A mixture of the two could not be separated when chromatographed on either calcium carbonate or deactivated alumina, using in each case light petroleum containing 5% (v/v) ether as developer. Furthermore, their absorption spectra over the range studied, 400–550 mμ., were identical in position and shape.

The traces of lemon-yellow pigment (fraction 6*A*, Table 2) could not be identified.

(*b*) *Quantitative experiment*

The relative amounts of the polyenes present in *Dacromyces stillatus* are recorded in Table 3. It will be seen that β-carotene is the most abundant

pigment with ζ-carotene next. Torulene is probably present to a greater extent than is indicated in the table, because it tends to be unstable on the column.

Table 3. *The relative composition of the polyene constituents of* Dacromyces stillatus

Polyene	% of total polyenes
Phytoene	Trace
Phytofluene	1·2
α-Carotene	7·0
β-Carotene	39·5
γ-Carotene	Trace (<1%)
ζ-Carotene	19·0
Unknown	Trace (<1%)
Torulene*	9·7
Cryptoxanthin	11·3
Unknown	Trace (<1%)
Zeaxanthin	12·3

* This figure is a lower limit for torulene.

DISCUSSION

The general pattern of carotenoid distribution in *Dacromyces stillatus* is very similar to that observed in other fungi (especially those, e.g. *Phycomyces blakesleeanus*, which have been examined in detail for minor constituents), in fruit (e.g. tomatoes), roots (e.g. carrots) and berries (e.g. honeysuckle). There exists in these sources (*a*) the major components of the constituent polyenes which are always the most unsaturated carotenoids, e.g. β-carotene, cryptoxanthin, and (*b*) the minor components which are members of the Porter-Lincoln series. This series consists of a number of open-chain polyenes, all derivatives of lycopene and differing one from the other by four hydrogen atoms. In *Dacromyces* two members of the series, neurosporene and lycopene, are apparently missing, but perhaps it would be unwise to assume that they would not have been detected in traces if much more material had been available to us. The significance of the general occurrence of the Porter-Lincoln series in the non-photosynthetic tissues and organisms and its complete absence from photosynthetic material is by no means obvious.

Porter & Lincoln (1950) consider that lycopene is synthesized in tomatoes (and presumably in other tissues now known to contain their series) by the stepwise removal of four hydrogen atoms from, respectively, tetrahydrophytoene, phytoene, phytofluene, ζ-carotene and neurosporene. This may well be so, but their further assumption that α- and β-carotenes are then produced by the isomerization of lycopene, is, according to recent work on tomatoes (Goodwin & Jamikorn, 1952) and on *Phycomyces* (Goodwin, Jamikorn & Willmer, 1952), probably incorrect, although traces may be formed in this way. There is therefore now no *a priori* reason for assuming that the major portion of the β-carotene found in photosynthetic tissues is produced by a route different from that occurring in non-photosynthesizing material, for the basis of this previously held view was that β-carotene synthesis in fruit occurred predominantly via the Porter-Lincoln series. The mode of synthesis of β-carotene in photosynthetic and non-photosynthetic tissues may be different, but it is well to emphasize that at the present moment we have no direct evidence for assuming such a difference.

SUMMARY

1. The following polyenes have been found to occur in the fungus *Dacromyces stillatus*: phytofluene, α-carotene, β-carotene, ζ-carotene, torulene, cryptoxanthin and zeaxanthin. β-Carotene is the major pigment present.

2. γ-Carotene and phytoene are also probably present in traces. Traces of two unidentified carotenoids have also been observed.

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REFERENCES

Bonner, J., Sandoval, A., Tang, Y. W. & Zechmeister, L. (1946). *Arch. Biochem.* **10**, 113.

Garton, G. A., Goodwin, T. W. & Lijinsky, W. (1951). *Biochem. J.* **48**, 154.

Goodwin, T. W. (1952*a*). *Biochem. J.* **50**, 550.

Goodwin, T. W. (1952*b*). *Biochem. J.* **51**, 458.

Goodwin, T. W. & Jamikorn, M. (1952). *Nature, Lond.* **170**, 104.

Goodwin, T. W., Jamikorn, M. & Willmer, J. S. (1952). *Biochem. J.* **53**, 531.

Goodwin, T. W. & Morton, R. A. (1946). *Analyst*, **71**, 15.

Haxo, F. (1949). *Arch. Biochem.* **20**, 400.

Kohl, F. G. (1902). *Untersuchungen über das Carotin.* Leipzig: Bornträger.

Lederer, E. (1938). *Bull. Soc. Chim. biol., Paris*, **20**, 611.

Porter, J. W. & Lincoln, R. E. (1950). *Arch. Biochem.* **27**, 390.

Zopf, W. (1890). *Die Pilze.* Breslau: Trewendt.