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CAROTENOID PIGMENTS OF THE NEMATODE-TRAPPING HYPHOMYCETE *ARTHROBOTRYS OLIGOSPORA* (FRES.)

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Abstract—*Arthrobotrys oligospora* was found to contain β- and γ-carotene, neurosporoxanthin and torulene; β-carotene being the most abundant and the other three pigments fairly equal in distribution. It is suggested that the pigment characteristics of the fungi in the nematophagous series may be an aid in elucidating their taxonomic affinities.

INTRODUCTION

Arthrobotrys oligospora (Fres.) is a nematophagous fungus usually considered taxonomically as a member of the family Moniliaceae in the Fungi Imperfecti.[1] The hyphae of this fungus have been described as hyaline,[2] but when seen *en masse* are frequently imbued with a faint pink tint. Cultures of an isolate obtained from soil and derived from a single conidial head were notable for their pronounced pink coloration and the material was considered well suited to an investigation of its pigments.

RESULTS

When the pigments in hexane were chromatographed on a magnesium oxide–celite column, using increasing concentrations of acetone in hexane as the developing solvent, the extract resolved into four distinct coloured zones (Table 1).

TABLE 1. CHROMATOGRAPHY OF *A. oligospora* PIGMENT EXTRACT ON A MAGNESIUM OXIDE-CELITE 1 : 1 (V/V) COLUMN

Fraction	Appearance	Eluting solvent (acetone in hexane)	Probable nature	% of total
1	Dark orange	100% hexane	β-Carotene	44
2	Light orange	2% acetone	γ-Carotene	17
3	Raspberry pink	10% acetone	Torulene (or celaxanthin)	20
4	Brownish orange	Alkaline methanol (0·5% KOH)	Neurosporoxanthin	19

Fraction 1. The dark orange zone was eluted from the column with hexane. Its absorption spectra in different solvents (Table 2) suggested that it was β-carotene. The fraction 1 carotenoid and authentic β-carotene extracted from *Phycomyces blakesleeanus*[3] were rechromatographed on a magnesium oxide–celite column and no separation occurred. This fraction was thus identified as β-carotene.

Fraction 2. This light orange zone was eluted with 2% acetone in hexane, its absorption spectra (Table 2) suggested that it was γ-carotene. When this fraction mixed with a pure

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sample of γ-carotene obtained from *Calendula officinalis*[4] was rechromatographed on an alumina column, they could not be separated. Fraction 2 was therefore γ-carotene.

Fraction 3. The absorption spectra of this raspberry pink band (eluted with 10% acetone in hexane) in various solvents (Table 2) suggested that it was either torulene or celaxanthin. When torulene obtained from *Rhodotorula rubra*[5] was mixed with fraction 3 and rechromatographed on a calcium hydroxide column using benzene as developer, there was no separation. Celaxanthin and torulene have very much the same absorption spectra in various solvents, but when "developed with benzene on calcium hydroxide, torulene is adsorbed well below celaxanthin ester and still further below celaxanthin".[5] As far as we know, celaxanthin has not been found in any fungus and the evidence points to the fact that fraction 3 was therefore torulene.

TABLE 2. ABSORPTION SPECTRA OF CAROTENOID FRACTIONS (IN VARIOUS SOLVENTS)

Carotenoid fraction	Wavelengths of maximal absorption (mμ)			
	Hexane	Benzene	Alkaline methanol (0·5% KOH)	Carbon disulphide
Total carotenoid fraction	⌒430, 455, 483, 515			
Fraction 1	⌒425, 452, 480	⌒437, 465, 493		⌒452, 483, 518
Authentic β-carotene	⌒425, 451, 482	⌒435, 465, 492		⌒451, 484, 520
Fraction 2	432, 462, 493	450, 475, 508		461, 495, 530
Authentic γ-carotene	434, 462, 494	449, 477, 510		463, 496, 532
Fraction 3	459, 486, 520	473, 501, 536		490, 522, 565
Authetic torulene	457, 485, 520	470, 502, 538		491, 525, 566
Fraction 4	⌒445, 470, ⌒500	⌒462, 488, ⌒510	435, 464, 500	⌒465, 495, ⌒525
Authentic neurosporoxanthin	⌒440, 472, ⌒505	⌒460, 486, ⌒515	432, 465, 500	⌒463, 496, ⌒523

⌒ denotes an inflexion.

Fraction 4. This brownish orange zone was the most strongly adsorbed and could be eluted by alkaline methanol (0·5% KOH). It then ran freely through the column and was rechromatographed on a sucrose column using 10% methanol–hexane as developer. Absorption spectra of this fraction in various solvents (Table 2) and its behaviour on the columns suggested that it might be the xanthophyll, neurosporoxanthin. Authentic neurosporoxanthin obtained from *Neurospora crassa*[6] and fraction 4 were co-chromatographed on a sucrose column and did not separate. This fraction was therefore identified as neurosporoxanthin.

Quantitative distribution. $3{\cdot}35 \times 10^{-2}$ per cent of the dry weight of the cultures was carotenoid and apart from β-carotene (44·3%) the three other pigments were fairly equal in distribution (Table 1).

DISCUSSION

Valadon[7] has suggested that if carotenoid pigments are to be used as a taxonomic characteristic, total spectra are not sufficient, and it is essential that one knows all the individual carotenoids present in a fungus on a well-defined medium and under strict cultural conditions. The present investigation has shown that *A. oligospora* contains β-

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and γ-carotene, torulene and neurosporoxanthin. The first two pigments are widely distributed among fungi and are found together in *Allomyces javanicus*, in *Cantharellus cibarius*[8] and in a large number of other species, but had not yet been found associated with torulene and neurosporoxanthin in the same organism. The nematode-trapping fungi may be a polyphyletic group although they exhibit morphological uniformity, and characters other than morphological may help in settling this question. An investigation of pigment characteristics of a large number of species could prove a useful means of doing so. It is hoped to report on such an investigation in the future.

EXPERIMENTAL

The fungus was grown in shake-culture in maize-meal extract broth (20 g/l. maize-meal) in the light and at room temperature (15–20° C). After a 15-day incubation period the material was removed for extraction of the pigments.

The cultures were filtered with a Buchner funnel, washed several times with distilled water, and extraction carried out by grinding the mycelium with methanol. The pigments were then transferred from the methanol extract to hexane.

Column chromatography was carried out using a mixture of magnesium oxide and celite (Light's Hyflo Super Cel) 1 : 1 (v/v), prepared by the A.O.A.C. method.[9] The pigment mixture was added to the column and development effected by washing with increasing concentrations of acetone in hexane. The absorption spectra of the carotenoids were measured in various solvents using a Unicam SP. 500 photoelectric spectrophotometer. The $E^{1\%}_{1cm}$ of β-carotene at 452 mμ was taken as 2560,[9] that of γ-carotene at 462 mμ as 2720,[9] that of torulene at 486 mμ as 2690[10] and that of neurosporoxanthin at 464 mμ as 2680.[6]

Pure β-carotene was isolated from *Phycomyces blakesleeanus* (–),[3] γ-carotene from the dark orange variety of the marigold (*Calendula officinalis*),[4] torulene from *Rhotodorula rubra*[5] and neurosporoxanthin from *Neurospora crassa*.[6]

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