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SHORT COMMUNICATION

THE CAROTENOIDS OF THE FUNGUS *PILAIRA ANOMALA*

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Abstract—The yellow–orange mycelium of *Pilaira anomala* (Ces.) Schroet. yielded about 420 μg of "carotene" per g dry weight, which separated into five pigment bands on alumina. The principal pigment was found to be β-carotene.

INTRODUCTION

Pilaira anomala (Pilobolaceae) has the characteristic of many other light-sensitive members of the Order Mucorales in that it produces carotenoids, both in vegetative hyphae and sporangiophores, when grown in the light. There are only brief references in the literature to this pigmentation. In 1934, Grove[1] stated that the spores were "yellowish in mass" and that the sporangium was "yellow at first". Schopfer[2] later found that under suitable nutritional conditions the mycelium contained "enormous quantities of carotene". In the related *Pilobolus*, the pigment has been described as "carotene",[3] but no complete analysis of these carotenoids has ever been published.

Other mucoraceous fungi on which recent investigations into their carotenoid pigments have been made, include: *Phycomyces blakesleeanus* Burgeff (Mucoraceae),[4] *Syzygites megalocarpus* Ehrenberg ex Fries (Mucoraceae)[5] and *Blakeslea trispora* Thaxter (Choanephoraceae).[6] The total coloured carotenoids of *Phycomyces* was found to be 95 per cent β-carotene, 2 per cent α-carotene, and 1 per cent γ-carotene; *Syzygites* was found to have 71 per cent β-carotene, 24 per cent γ-carotene and 5 per cent lycopene; and *Blakeslea* was found to have 53 per cent β-carotene, 27 per cent γ-carotene and 14 per cent lycopene. Some very high yields of carotenoids have been reported with these fungi: *Phycomyces* produced more than 3000 μg/g,[7] Wenger and Lilly[5] found that *Syzygites* produced 600 μg/g and Sutter and Rafelson[8] obtained 2189 μg/g with mated strains of *Blakeslea.*

RESULTS

The pigment extract from *Pilaira anomala* was separated from lipids on a column of Sephadex LH-20. Purity was checked at this stage by i.r. spectra analysis. The pigment was

1 W. B. GROVE, in *Researches in Fungi* (edited by H. R. BULLER), Vol. VI, Longmans Green, New York (1934).
2 W. H. SCHOPFER, *Protoplasma* **31**, 195 (1938).
3 E. BÜNNING, *Planta* **26**, 719 (1937).
4 T. W. GOODWIN, *Biochem. J.* **50**, 550 (1952).
5 C. J. WENGER and V. G. LILLY, *Mycologia* **58**, 671 (1966).
6 D. M. THOMAS and T. W. GOODWIN, *Phytochem.* **6**, 355 (1967).
7 V. G. LILLY, H. L. BARNETT and R. F. KRAUSE, *Proc. W. Va. Acad. Sci.* **29**, 25 (1957).
8 R. SUTTER and M. E. RAFELSON, *J. Bacteriol.* **95**, 426 (1968).

resolved into five fractions on a column of alumina (Table 1). Fraction A representing 8 per cent of the total pigment had one sharp peak and a "shoulder" on one side. Fraction B representing 71 per cent of the total pigment was identified as β-carotene, both from spectra in different solvents[9] and from co-chromatography with an authentic sample in different solvent systems on loaded papers. Fraction C at 1 per cent was too small to be identified. Fraction D representing 16 per cent of the total pigment had one intense spectral peak with a prominent "shoulder" on either side and Fraction E, some 4 per cent of the total with two small peaks, almost forming a plateau, and a third intense peak.

The yellow–orange pigments remained in the epiphase when the cyclohexane solution was shaken with an equal volume of 90% aqueous methanol, indicating the probable absence of unesterified and unmethylated xanthophylls. Therefore fractions A, D and E are thought to be carotenes other than β-carotene.

The total quantity of "carotene" pigment present (related to β-carotene) was 420 μg/g dry mycelium, which principally consisted of vegetative hyphae.

TABLE 1. THE CHROMATOGRAPHIC SEPARATION OF THE CAROTENES OF *Pilaira anomala* ON ALUMINA

Fraction	Solvent (v:v)	Colour	Absorption maxima (nm) in cyclohexane			% of total pigments
A	Ether:cyclohexane (1:99)	Yellow		445	470	8
B	Ether:cyclohexane (2:98)	Orange	420	452	480	71
C	Ether:cyclohexane (10:90)	Yellow	Undetectable			1
D	Ether:cyclohexane (20:80)	Orange	430	460	490	16
E	Ether	Pink	400	420	465	4

EXPERIMENTAL

Cultural Conditions

Author's isolate of *Pilaira anomala* from deer dung (Bushy Park, Middlesex)* was cultured on: glucose 1 per cent; vitamin-free casamino acids (Difco) 0·5 per cent; yeast extract (Difco) 0·05 per cent; salts (Page,[10] 1952). 100 ml medium, adjusted to pH 7·2 with KOH, were used in 1 l. Roux bottles, laid flat in the incubator. They were inoculated with a heavy spore suspension from an agar slope of the same medium and incubated at 25° in continuous light at 750 lux for 9 days. Four replicates were set up.

Extraction

Filtered mycelium was ground to a paste with acid-washed sand in 5 ml diethyl ether. An equal volume of anhydrous Na_2SO_4 was added and thoroughly mixed with the ground mycelium. The ground material was extracted on a vacuum pump with diethyl ether until extracts were colourless. The extracts were bulked and concentrated. The concentrate was placed on a Sephadex LH-20 column (250 × 8 mm); five 2-ml coloured fractions were taken from the Sephadex column, each was transferred to 250 × 8 mm alumina column and eluted as five bands with increasing quantities of diethyl ether in cyclohexane.

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* Deposited as C.M.I. 109387 at Commonwealth Mycological Institute, Kew.

[9] B. H. DAVIES, in *Chemistry and Biochemistry of Plant Pigments* (edited by T. W. GOODWIN), Academic Press, New York (1965).

[10] R. M. PAGE, *Am. J. Botany* **39**, 731 (1952).