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SHORT COMMUNICATION

THE PRESENCE OF β-ZEACAROTENE IN CULTURES OF DIPHENYLAMINE-INHIBITED *PHYCOMYCES BLAKESLEEANUS*

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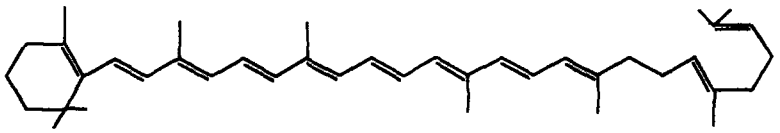
Abstract—Pigment "X" reported in diphenylamine-inhibited cultures of *Phycomyces blakesleeanus* in 1953 by Goodwin and Osman has been identified as β-zeacarotene. The original material also contained traces of ζ-carotene.

IN 1952 Goodwin[1] found that diphenylamine (DPA) specifically inhibited synthesis of β-carotene in *Phycomyces blakesleeanus* and caused the accumulation of phytoene, phytofluene, ζ-carotene and, to a lesser extent, neurosporene. Associated with ζ-carotene was an unidentified pigment carotene "X".[2] Later Petzold *et al.*[3] isolated from maize a new pigment, β-zeacarotene; they proposed the structure 7',8'-dihydro-γ-carotene (I) and this was confirmed by total synthesis.[4] Its general properties suggested that it might be identical with carotene "X". Their identity has now been established.



(I)

Five conical flasks (250 ml) containing liquid medium (50 ml) to which had been added diphenylamine,[1] were inoculated with a spore suspension of *P. blakesleeanus*. After 7 days' growth the mats were harvested and the unsaponifiable fraction extracted by standard methods[5] and dissolved in light petroleum (b.p. 40–60°). Sterols were precipitated from the unsaponifiable matter by cooling to −20°. The sterol-free solution was chromatographed on a column of $Ca(OH)_2$: Celite (3:1, w/w) with light petroleum (b.p. 40–60°). Five fractions were obtained; the first was eluted, whilst the others were obtained by extruding the column and cutting out the coloured zones. After further purification *fraction* 1 was separated into two polyenes identified as phytoene and phytofluene, and *fractions* 2, 4 and 5 were shown to be β-carotene, ζ-carotene, and neurosporene, respectively. *Fraction* 3 had absorption

[1] T. W. GOODWIN, *Biochim. J.* **50**, 550 (1952).
[2] T. W. GOODWIN and H. G. OSMAN, *Arch. Biochem. Biophys.* **47**, 215 (1953).
[3] E. N. PETZOLD, F. W. QUACKENBUSH and M. MCQUISTAN, *Arch. Biochem. Biophys.* **82**, 117 (1959).
[4] R. RÜEGG, U. SCHWIETER, G. RYSER, P. SCHUDEL and O. ISLER, *Helv. Chim. Acta* **44**, 994 (1961).
[5] T. W. GOODWIN, *Modern Meth. Plant Anal.* **3**, 372 (1955).

maxima (379, 399, 424, 454 mμ in light petroleum, b.p. 40–60°) similar to carotene "X".[2] Part of the fraction was chromatographed on a Kieselgel plate with authentic all *trans*-β-zeacarotene (405, 428, 455 mμ, light petroleum) and ζ-carotene; the fraction separated into zones corresponding to the two authentic pigments.

The same result was obtained with the main portion of *fraction* 3 on a column of Kieselgel G with light petroleum (b.p. 60–80°) as developer. Two bands eventually separated; the first appeared to be β-zeacarotene, whilst the slower moving band was identified as ζ-carotene. Thin-layer chromatography of the first band together with authentic β-zeacarotene and iso-β-zeacarotene (7,8-dihydro-γ-carotene) on alumina with light petroleum (b.p. 60–80°) as developer showed that the *Phycomyces* pigment ran with β-zeacarotene (R_f 0·82) and easily separated from iso-β-zeacarotene (R_f 0·46). The absorption spectrum of the β-zeacarotene fraction was also identical with that of authentic all *trans*-β-zeacarotene.[4,6] It is clear that carotene "X" is β-zeacarotene and that the original preparation also contained traces of ζ-carotene.

The presence of β-zeacarotene in DPA-inhibited *Phycomyces* when β-carotene synthesis is blocked but polyenes earlier in the pathway accumulate, lend support for the idea that the formation of β-carotene proceeds from the primary 40-C compound phytoene via the acyclic intermediates, phytofluene, ζ-carotene and neurosporene, and that the latter cyclizes to β-zeacarotene which is then transformed, possibly via γ-carotene into β-carotene. Other workers have also recently produced evidence in support of this view.[7]

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[6] K. L. SIMPSON and T. W. GOODWIN, *Phytochem.* 4, 193 (1965).
[7] K. L. SIMPSON, T. O. M. NAKAYAMA and C. O. CHICHESTER, *Abstr. Fed. European Biochem. Soc., London* p. 57 (1964).