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SHORT COMMUNICATION

# THE CAROTENOIDS OF THE FUNGUS *EPICOCCUM NIGRUM* LINK

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**Abstract**—From the dark-red coloured mycelium of *Epicoccum nigrum* Link four carotenoid pigments were isolated. Three of them were identified as β-carotene, γ-carotene and torularhodin. The fourth one with great probability seems to be rhodoxanthin, a keto-carotenoid never found so far as a fungal metabolite.

## INTRODUCTION

THE knowledge of the chemical structure of pigments is an important tool for the taxonomic identification of fungi, particularly in the case of closely related species. In the present paper the presence of carotenoid pigments is demonstrated in *Epicoccum*, of which about seventy species are supposed to exist.

Several authors have investigated on the pigments of this fungus but so far the existence of carotenoids has not been mentioned. Naumann[1] in 1912 extracted a red pigment from *Epicoccum purpurascens* Ehrenberg and observed the colour variation under acidic and alkaline conditions. Moreau and Moreau,[3] studying the same pigment, made quite different observations. In 1959 Schol-Schwarz[2] showed that different species could be unified into the single species *E. nigrum* Link, with the exception of *E. andropogonis* (Ces.) Schol-Schwarz formerly known as *Cerebella andropogonis* (Ces.).

Finally Bamford *et al.*[4] isolated a yellow pigment from both *E. nigrum* Link and *E. andropogonis* (Ces.) Schol-Schwarz, which was identified as 3,4,5-trihydroxy-6-methyl-phthalaldehyde.

## RESULTS

The orange-red pigment extract was separated into four fractions on a cellulose column. Each fraction was rechromatographed on an alumina column.

Fraction A, which represents about 30 per cent of the total pigment, has all the properties of β-carotene. Its identity with β-carotene was determined by mixed chromatography with an authentic sample on cellulose and alumina columns.

Fraction B showed absorption spectra in different solvents identical with those of γ-carotene. Co-chromatography with an authentic sample confirmed this identity.

[1] K. W. NAUMANN, *Hedwigia* **51**, 135 (1912).
[2] M. B. SCHOL-SCHWARZ, *Trans. Brit. Mycol. Soc.* **42**, 149 (1959).
[3] C. MOREAU and M. MOREAU, *Bull. Soc. Linneenne Normandie* **6**, 71 (1951).
[4] P. C. BAMFORD, G. L. F. NORRIS and G. WARD, *Trans. Brit. Mycol. Soc.* **44**, 354 (1961).

Fraction C, representing about 40 per cent of the pigments, seems to be rhodoxanthin, 3,3'-diketo-retro-β-carotene. It had the same spectra in different solvents as listed by Goodwin[5] for rhodoxanthin. The wine-red pigment in ethanol (95 per cent) was reduced by sodium borohydride to a yellow compound, which appears identical with eschscholtzxanthin, 3,3'-dihydroxy-retro-β-carotene. The dioxime, prepared by reaction of the pigment with hydroxylamine in a boiling solution of pyridine–ethanol under nitrogen, showed the same spectra as given by Kuhn and Brockmann[6] for the expected product. Co-chromatography with an authentic sample of rhodoxanthin on alumina and cellulose columns and thin-layer chromatography showed a close relationship between the two pigments.

Fraction D could be identified by its very strong adsorbence on Florisil and alumina columns as an acidic carotenoid. Its absorption spectra in different solvents were identical with those reported by Karrer and Rutschmann[7] for torularhodin; especially the shift of the spectrum in carbon disulphide. Specific colour reactions for torularhodin gave positive results. The methyl ester, obtained by addition of diazomethane in hexane at 5°, showed the same properties as torularhodin methyl ester.

It should be emphasized that this is the first time that a ketocarotenoid with these characteristics has been isolated as a fungal metabolite.

TABLE 1. THE CHROMATOGRAPHIC SEPARATION OF THE FOUR CAROTENOIDS OF *E. nigrum* Link

| Fraction* | Solvent for elution† | Colour | Absorption max (nm) in hexane | Relative abundance, of % total | Identification |
|---|---|---|---|---|---|
| A | H | Yellow | (426), 453, 482 | 32 | β-Carotene |
| B | H:A (99:1) | Orange | 438, 463, 495 | 22 | γ-Carotene |
| C | H:A (95:5) | Red | 458, 489, 524 | 40 | Rhodoxanthin (?) |
| D | H:A (90:10) | Violet | 468, 501, 536 | 6 | Torularhodin |

* Adsorbent cellulose powder; pigments in order of increasing adsorptivity.
† H=hexane; A=acetone.

## EXPERIMENTAL

*Solvents.* The solvents used were obtained from Carlo Erba, Milan, Italy, and were all of chromatographic grade. The diethyl ether was freed from peroxides immediately before use.

*Chromatographic adsorbents.* Alumina (Brockmann, activity 1) was obtained from E. Merck A.G., Darmstadt, Germany; Cellulose powder (standard grade) from W. & R. Balston Ltd., England, and Florisil (100/200 mesh) from the Floridin Company, Pennsylvania, U.S.A.

*Other chemicals.* Synthetic β-carotene and rhodoxanthin were obtained from F. Hoffmann-LaRoche Ltd., Basle, Switzerland. Pure γ-carotene was extracted from carrots.

*Organism and cultural conditions.* A strain of *Epicoccum nigrum* Link was received from Professor E. Küster, Department of Industrial Microbiology, University of Dublin, Ireland. The medium used contained glucose 1% and yeast autolysate 0·5%, pH 6·8–7·0. The cultures were grown in 500-ml. Erlenmeyer flasks containing 100 ml of medium, put on a rotatory shaker at 220 rev/min continuously illuminated for 3 days at 24°.

*Extraction and chromatography.* After filtration the mycelium was extracted exhaustively with acetone in a Waring Blendor and the extract containing the pigments was then transferred into hexane. The pigments were chromatographed on a 1·5 × 20 cm cellulose column and developed using hexane with an increasing quantity of acetone. Four fractions were separated. Fractions A, B and C were rechromatographed on 1 × 15 cm alumina

[5] T. W. GOODWIN, In *Chemistry and Biochemistry of Plant Pigments*. Academic Press, New York (1965).
[6] R. KUHN and H. BROCKMANN, *Chem. Ber.* **66**, 828 (1933).
[7] P. KARRER and J. RUTSCHMANN, *Helv. Chim. Acta* **26**, 2109 (1943); *Helv. Chim. Acta* **28**, 295 (1945); *Helv. Chim. Acta* **29**, 355 (1946).

columns and developed with hexane containing increasing amounts of acetone. Fraction D was rechromatographed on a 0·5 × 10 cm alumina column with hexane–glacial acetic acid (99·5 : 0·5 v/v). Sterols were eliminated by precipitation, standing for 48 hr at −20° in the dark.

*Apparatus.* For determining the absorption spectra a Cary spectrophotometer Model 11 MS-50 was used.

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