

## A Note on Properties of Pigments Produced by *Epicoccum nigrum*

The fungus *Epicoccum nigrum* when cultured under suitable conditions produces a red pigment which diffuses readily into the medium. There seems to be no agreement among investigators on the chemical nature and properties of the pigment. It has been referred to as flavipin, humic acid, caratenoids and flavinoids by various investigators<sup>1-4</sup>. In this paper some of the properties of the extracted crude pigment are reported.

**Materials and methods.** The microorganism used was *Epicoccum nigrum* (Strain 5-1-3)<sup>5</sup>, the inoculum consisted of a suspension of spore and mycelial fragments, the medium was the Czapek Dox glucose medium. The fungus was grown as surface felt and were incubated at 25°C exposed to 40–50 foot candles of artificial light from day light fluorescent electric tubes TL 80W/55, 5' suspended at a suitable distance above the culture vessels. The pigment was extracted from the acidified or non-acidified medium using suitable solvents including diethyl-ether, alcohol-ether and alcohol.

**Results. Solubility of the pigment.** The pigment produced by *E. nigrum* was found to be soluble in water, methanol, ethanol, ether and acetone. It was sparingly soluble in butanol, chloroform and ethyl acetate. It was insoluble in butyl methyl ketone, benzene and toluene.

**Chemical tests.** Various tests were performed on aqueous and alcohol-ether solutions of the pigment. A yellow pigment was obtained on treatment of the pigment with excess alkaline or excess acid. The pigment gave with aqueous solutions of ferric chloride an intense blue-black colour which darkened on standing. With Brady's reagent (0.32% 2, 4, Dinitrophenyl-hydrazine in 2N HCl) it gave an immediate red precipitate. It reduced ammoniacal silver nitrate and Fehlings solution on heating.

**Homogeneity of the pigment.** To test for homogeneity of the crude pigment both paper and thin-layer chromatography were carried out. The solvents used was water saturated butanol-acetone – 0.5N HCl (12:12:1.5). Using this solvent both paper chromatography and thin-layer chromatography of an alcohol solution of the red pigment revealed the presence of red, orange and yellow fractions in varying proportions.

**Absorption spectrum of the crude pigment.** Alcohol solution of ether extractable pigment was used and this showed an absorption maximum in the blue violet region of the spectrum with peaks of 330–350 mU. In addition to absorption in the blue violet region, the solution showed a secondary absorption maximum in the red end of the spectrum with peaks of 1000–1010 mU.

**Discussion.** The findings here tend to support the presence of flavipin-like substance in the crude pigment secreted by *E. nigrum*. It is also possible that carotenoids, flavinoids and humic acid-like substance are also elaborated by the fungus. In studying the pigment produced by *E. nigrum* various investigators have followed different lines of approach and it is possible that each may be dealing with a fraction of what appears to be a complex substance. Further investigations to establish the true chemical nature and other properties of the pigment may be rewarding.

**Zusammenfassung.** Untersuchungen der Pigmenteigenschaften des Pilzes *Epicoccum nigrum* ergaben die Anwesenheit einer flavipinartigen Substanz (3,4,5-trihydroxy-6-Methylphthal-Aldehyd).

O. U. EKA

Department of Industrial Microbiology, University College, Dublin 4 (Ireland), 18 February 1970.

- <sup>1</sup> P. C. BAMFORD, G. NORRIS and G. WARD, Trans. Br. mycol. Soc. 44, 354 (1961).
- <sup>2</sup> F. H. FOPPEN and O. GRIBANOVSKI-SASSU, Biochem. J. 106, 97 (1968).
- <sup>3</sup> J. P. MARTIN, S. J. RICHARDS and K. HAIDER, Soil Sci. Soc. Am. Proc. 37, 657 (1967).
- <sup>4</sup> O. U. EKAH, Ph. D. Thesis National University of Ireland (1969).
- <sup>5</sup> B. SCHOL-SCHWARZ, Trans. Br. mycol. Soc. 42, 149 (1959).

## STUDIORUM PROGRESSUS

### Comparative Pharmacopsychological Study of the Effects Produced by Psychopharmaceuticals on Verbal Interaction in a Group of Students

**1. Statement of the problem.** Pharmacopsychological research aims at establishing criteria by which the influence on human experience and behaviour of substances affecting the CNS can be reliably described in its most important dimensions. Both a review of the methods used in pharmacopsychology, such as JANKE<sup>1</sup> produced, and the study of newer papers in this field, reveal that the effects of psychotropic substances on the individual's social behaviour have been most inadequately investigated.

This does not apply to the clinical and therapeutic use of psychopharmaca in which target symptoms (FREYHAN<sup>2</sup>) such as 'affectivity', 'social adjustment', 'contact with other patients', and 'aggression' form a part of every psychiatric observation or interview. Above all, the deficiency makes itself felt in work with normal subjects,

which might be expected to assess the pattern of effects (specific psychological effects) produced by the psychotropic drug under controlled conditions. The following questions must, therefore, be asked of pharmacopsychological studies: (1) Is it possible to produce a method of assessing the effects which psychotropic substances produce on the social behaviour of clinically normal individuals? (2) Can the effects of substances which are pharmacologically and clinically distinguishable be differentiated from one another and from placebos with regard to the dimensions described?

**2. Method.** We developed the following experimental design for clarifying these questions: Two clinically and pharmacologically distinguishable substances were given in random succession to student volunteers who were taking part in a psychoanalytically oriented personal ex-