On the Biosynthesis of the Pigments of *Penicillium islandicum* III

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In the studies of the biosynthesis of anthraquinones, approximately 50 UV-induced mutants of *Penicillium islandicum* Sopp N.R.R.L. 1036 have been isolated, in the hope that some of the mutants would accumulate an intermediate metabolite because a step in the reaction chain leading to the formation of anthraquinones had been blocked by mutation. The mutants investigated were selected on the basis of their visually abnormal pigment production as compared with the wild type, and a series of strains was obtained varying in colour from pigment-free to dark brown. Paper chromatographic analysis of the pigments of the mycelia and of the aromatic substances occurring in the culture media showed that only variations with respect to the quantities of the normally produced individual substances had been induced by the UV treatment. In the pigment-free mutants no aromatic compounds were detectable with the reagents used.

The principal anthraquinone pigments produced by *P. islandicum* as skyrin, rubroskyrin, iridoskyrin and islandicin, are formed from acetate units by head to tail couplings [1,2] and, despite their close structural relationships, they are not interconvertible *in vivo* but seem to be derived from a common prearomatic stage. The failure to block the formation of any one of these pigments by mutation is in accordance with related findings, published elsewhere [3]. Evidently, the only way to effect a change of the aromate production is to interfere with the acetate condensing enzyme(s) resulting in a general blocking of the aromate production. Consequently, the biogenesis of the anthraquinones should not take place by stepwise formations of defined intermediates such as benzene derivatives, but be dependent throughout on the participating of activated acetate. The increased production of individual pigments has been utilized in investigations published previously [4,5].

Experimental. *Preparation of the mutants.* The culture media used were Czapek-Dox solution (minimal medium) and Czapek-Dox solution supplemented with hydrolyzed casein, B-vitamins, nucleic acid hydrolyzate, Difco malt extract and yeast extract (complete medium). Both media were adjusted to pH 4.5 and 2.5 % Difco agar was added. A suspension of condiaspores in sterile, distilled water was irradiated with a Westinghouse steril-lamp, and then filtered through sterilized filter paper to remove spore clusters and mycelia fragments. The irradiation was done in a small, open petri dish with 5 ml of spore suspension with 10^5 spores per ml. The distance from the UV-lamp was 11.5 cm and the spores were irradiated for 90 sec. After dilution to 10^4 spores per ml, 0.1 ml of the suspension was plated on each of 50 petri dishes containing complete medium. The untreated control was plated on 10 petri dishes, 50 spores per plate. After having grown for 6 days at 28°C, the colonies on the plates were counted, and it was found that the irradiated plates showed, on the average, 1 % survival of the conidiaspores. Among the treated colonies, 50 were selected, which on visual inspection showed an abnormal pigmentation as compared with the wild type. They were transferred to slants of complete medium and each strain was re-isolated by plating on complete medium. From a single colony, spores were transferred to minimum as well as to complete medium to decide whether some of the strains showed different pigmentation when cultivated on the two media. This was not the case.

All the re-isolated strains were grown in culture flasks containing 150 ml of Czapek-Dox liquid medium, two flasks for each strain. These cultures were utilized in the chemical investigations described below.

Chemical analysis of the mutants. The cultures were harvested after two and three weeks' growth, respectively, and the mycelia were separated from the media by filtration. Each mycelium was mechanically disintegrated in 25 ml of acetone, and after centrifugation, 0.5 ml of the supernatant was developed on a paper chromatogram with benzene-formic acid (2 %) (10:1) as the solvent. The pigments were recognized by their R_F values and colour reactions with alcoholic magnesium acetate.

	Skyrin	Rubro-skyrin	Irido-skyrin	Islan-dicin
R_F	0.15	0.25	0.90	0.95
			blue	blue
Colour	violet	green	violet	violet

The relative quantities of the different pigments were visually estimated by a direct comparison with the amounts produced by the wild type.

The culture medium was extracted with ether, the residue after evaporation redissolved in acetone, and one third of the solution used for paper chromatographic analysis. After running in a solvent consisting of chloroform-methanol-formic acid (4 %) (10:1:1) the paper was sprayed with alcoholic ferric chloride, dibromoquinone chloroimide, diazotized sulfanilic acid and phosphomolybdic acid. The culture media of the mutants, with the exception of the pigment-free ones, contained in varying amounts the aromatic compounds normally produced by the wild type.

The only difference observed between the cultures grown for three weeks and two weeks, respectively, was a higher production of the substances investigated in the former.

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A Note on Proton Magnetic Resonance Spectra of Alcohols in Carbon Tetrachloride

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In the high resolution proton magnetic resonance (PMR) spectra of the pure forms of most primary and secondary alifatic alcohols, the signal from the OH-protons are obtained as multiplets. When such an alcohol, *e.g.* ethanol, is diluted with a nonpolar solvent such as *cyclo*hexane or *per*--deuterocyclohexane, the OH-triplet is found to persist down to concentrations where the noise level no longer permits observations. The addition of small amounts of carbon tetrachloride to pure ethanol, however, changes the triplet into a sharp singlet [1]. This is rather unexpected since carbon tetrachloride is generally assumed to be about as nonpolar as *cyclo*hexane. We have, however, found that if commercially available carbon tetrachloride of *pro analysi* quality, which in mixtures with ethanol produces OH-singlets, was first washed with a 0.01 M solution of sodium hydroxide and then with three or four portions of water and thereafter kept away from direct or indirect sunlight, the addition of this product to a purified alcohol, *e.g.* ethanol, did not affect the splitting of the OH-signal, *i.e.* this signal was displayed as a triplet down to concentrations where the noise level no longer permitted direct observations (mole fraction alcohol *ca.* 0.02). In Fig. 1 the PMR spectrum at mole fraction alcohol 0.1 is shown (*cf.* PMR spectrum in Ref.[1], p. 3).

Exposure of a mixture of ethanol and purified carbon tetrachloride (mole fraction alcohol 0.8) to ultraviolet light or sunlight 5—30 sec. was, however, found to be sufficient to produce a sharp OH-singlet from the triplet. The extreme sen-

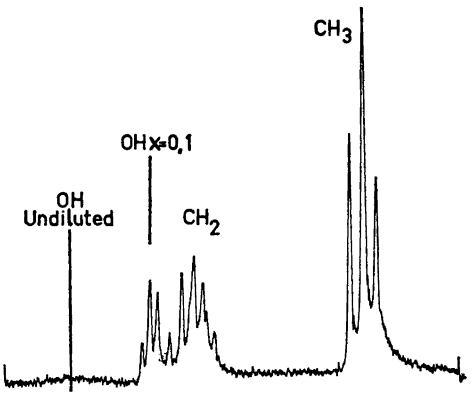

Fig. 1. PMR spectrum of ethanol in carbon tetrachloride purified according to text. Mole-fraction ethanol = 0.1. The position of the OH-triplet in pure ethanol is indicated (*cf.* spectrum in Ref.[1], p. 3).

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