STUDIES RELATED TO THE BIOSYNTHESIS OF PRODIGIOSIN IN SERRATIA MARCESCENS

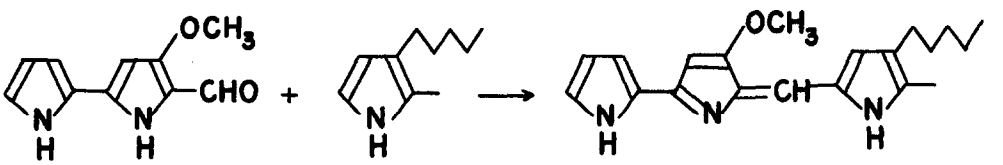
Harry H. Wasserman, James E. McKeon and Ursula V. Santer

The Departments of Chemistry and Microbiology,
Yale University, New Haven, Connecticut

Received July 29, 1960

The possibility that the biosynthesis of prodigiosin may be related to the mechanism of porphyrin formation was proposed a number of years ago by Hubbard and Rimington (1950) who found that, as in heme biosynthesis, both acetate and glycine are incorporated into prodigiosin. However, Marks and Bogorad (1960) have recently reported that δ-aminolevulinic acid-5-C^{14} is not utilized by Serratia marcescens in the formation of prodigiosin, indicating that the red pigment is derived from a source other than porphobilinogen, the pyrrolic precursor of porphyrin.

In another study on the biosynthesis of prodigiosin, Santer (1958) has isolated a $C_{10}H_{10}O_2N_2$ pyrrole-containing precursor of prodigiosin from a mutant strain of Serratia (9-3-3) which does not form prodigiosin (Santer and Vogel, 1956). As outlined in a preliminary communication (Wasserman et al., 1960) the properties of the C-10 precursor are best in accord with the α,α'—dipyrrole aldehyde structure I, while prodigiosin, formed by acid-catalyzed condensation of methylamylpyrrole (II) with the aldehydic precursor has been formulated as the dipyrrylmethene III.



I II III

Prodigiosin is also formed by strain 9-3-3 when the mutant is exposed to a substance excreted by strain W-1 which cannot form I (Santer and Vogel, 1956). The substance formed by strain W-1 is volatile and is most probably methylamylpyrrole.

We have now found that when twenty-four hour cultures of Serratia marcescens, 9-3-3 (from which the $C_{10}H_{10}O_2N_2$ precursor can be isolated) are exposed to the vapors of pure synthetic methylamylpyrrole, prodigiosin formation takes place within minutes.

The methylamylpyrrole was prepared by an unambiguous synthetic route from 2-oximinooctan-3-one and ethyl oxaloacetate, followed by decarboxylation, and was identical (superimposable infrared spectra) with the $C_{10}H_{17}N$ pyrrole obtainable by alkaline degradation of prodigiosin (Wrede and Rothhaas, 1934). The 9-3-3 Serratia strain was grown in trays on 2% agar gel containing 1% glycerol and 0.5% Bacto-peptone. After twelve hours the trays were inverted over strips of filter paper impregnated with methylamylpyrrole, the culture surface being about 1 cm. above the pyrrole-containing paper. Color development on the agar was observed almost immediately, and after thirty-six hours the pigment was harvested from the agar. Isolation of prodigiosin was accomplished by treatment of the cellular material with 10% potassium hydroxide followed by exhaustive extraction with methylene chloride, and then chromatography on alumina (Fisher 80-200 mesh). The free base obtained from the benzene-ether eluate was converted to the perchlorate which was purified by recrystallization from ethanol-water. The infrared spectrum of the perchlorate salt in KBr disc is indistinguishable from the spectrum of analytically pure authentic prodigiosin perchlorate (Morgan and Tanner, 1955). The above findings suggest that prodigiosin synthesis in vivo occurs through a coupling stage similar to that observed

in the in vitro dipyrrylmethene condensation, I + II ⟶ III. We have also found (work to be published) that other pyrroles such as 2, 4-dimethylpyrrole can likewise be directly utilized by the 9-3-3 strain in the formation of analogues of prodigiosin, differing from III only in the nature of the alkyl-pyrrole fragment.

Other workers (Dietzel, 1949; Nicolaus, 1958; Bohlmann, 1960) have recently reported the isolation of prodigiosin-like materials from bacterial cultures. These pigments, having the composition $C_{25}H_{35}ON_3$ differing by five carbon atoms from III ($C_{20}H_{25}ON_3$), are most probably all the same substance. The extra five carbon atoms of this C-25 prodigiosin appear to be incorporated into the side chain of the pyrrole ring corresponding to methylamylpyrrole (Bohlmann, 1960).

We (HHW and JEM) are glad to acknowledge support from Grants P64 and P64A, of the American Cancer Society. We are grateful to Professor David M. Bonner for helpful discussions.

References

Bohlmann, F., private communication.

Dietzel, E., Z. physiol. Chem. 284, 262 (1949); Naturwissenschaften 35, 345 (1948).

Hubbard, R. and C. Rimington, Biochem. J. 46, 220 (1950).

Marks, G. S. and L. Bogorad, Proc. Natl. Acad. Sci., U. S. 46, 25 (1960).

Morgan, E. N. and E. M. Tanner, J. Chem. Soc. 3305 (1955).

Nicolaus, R. A., R. Nicoletti and F. Arcamone, Ricerca sci. 28, 2314 (1958).

Santer, U. V., Ph.D. Dissertation, Yale University (1958).

Santer, U. V., and H. J. Vogel, Biochim. Biophys. Acta 19, 578 (1956);

Federation Proc. 15, No. 1, Part I, 345 (1956).

Wasserman, H. H., J. E. McKeon, L. Smith and P. Forgione, J. Am. Chem. Soc. 82, 506 (1960).

Wrede, F. and A. Rothhaas, Z. physiol. Chem. 226, 95 (1934).