NORPRODIGIOSIN: OCCURRENCE IN A MUTANT OF SERRATIA MARCESCENS

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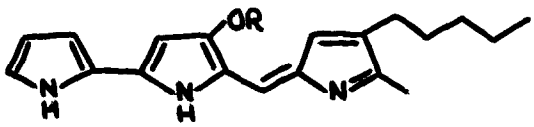
The structure of prodigiosin (I), the blood-red pigment of Serratia marcescens, has been established (Wasserman, 1960) and confirmed by synthesis (Rapoport and Holden, 1960). We now wish to present evidence that the principal pigment of an orange mutant of S. marcescens has structure II, differing from the wild-type pigment only in replacement of the methoxyl group by hydroxyl. In line with the nomenclature of other desmethyl natural products, we have designated this compound "norprodigiosin."

I. R = CH_3; Prodigiosin

II. R = H; Norprodigiosin



The orange mutant, S. marcescens strain OF, isolated by Williams and Green (1956) after irradiation of strain Nima, has been grown in our laboratories in large quantities for several years; we have made many unsuccessful attempts to isolate a crystalline orange pigment derivative (Burgus, 1960). Although prodigiosin itself readily forms a crystalline perchlorate salt (Wrede and Hettche, 1929), none of our chromatographic column fractions of the orange mutant pigment extract has ever shown any tendency to form a crystalline perchlorate. Rapid changes in solubility and color characteristics of these column fractions were suggestive of oxidation or polymerization reactions, some apparently light-catalyzed. We suspected that a labile functional group on the orange pigment was responsible for our difficulties in isolation work; since analyses for

methoxyl on crude fractions were negative, we assumed that we might be dealing with the hydroxy analog of the wild-type pigment. A methylation study was therefore carried out on crude mutant pigment.

For pigment production the culture medium of Williams, Green, and Rappoport (1956) was used. Generally the orange organism was grown in 9 x 14 in. borosilicate glass baking dishes, each containing 400 ml of agar medium; after covering with heavy aluminum foil, autoclaving, and inoculating with 10 ml of a 24-hour, 27° broth culture, the dishes were incubated in the dark at 27°. At the end of 5 days the cells were scraped from the surface, lyophilized, and stored in a deep-freeze until needed; yield averaged 1.3 g dry weight per dish. Water was added to the dry cells to make a paste and the pigment extracted with acetone (Williams, Green, and Rappoport, 1956) and later transferred into chloroform (Efimenko, et al., 1956). The chloroform was evaporated and the crude orange residue dissolved in diethyl ether and treated with an excess of diazomethane. Counter-current distribution of the methylated pigment for 20 transfers in a solvent system composed of 4 vols. petroleum ether (b.p. 65-67°), 3 vols. 2-methoxyethanol, and 1 vol. 0.01 M phosphate buffer, pH 7.2, revealed a new peak of material (followed by absorbancy at 540 mμ after acidification) having K = 0.82, identical to that of authentic prodigiosin in this solvent system.

The methylated orange pigment was washed with petroleum ether and the petroleum ether-soluble fraction chromatographed on acid alumina, pH 5-6, Brockman activity VI, using petroleum ether containing 0.25 percent absolute ethanol as the developing solvent. A bright red band separating was eluted to give an oily residue which was dissolved in a little hot 95 percent ethanol; addition of 5 percent aqueous perchloric acid yielded a crystalline perchlorate whose melting point of 223.5-224.5° was not depressed by admixture with an authentic sample of pro-

digiosin perchlorate. Analysis for $C_{20}H_{26}N_3O_5Cl$:

Found:	C, 56.68;	H, 6.14;	N, 9.98;	Cl, 7.95
Calculated:	C, 56.67;	H, 6.18;	N, 9.91;	Cl, 8.36

The free base obtained from the crystalline perchlorate of methylated orange pigment has absorption spectra in the IR, visible, and UV regions identical to those of prodigiosin reported by Castro, et al. (1959) and observed in our investigations. Behavior of the free base on chromatography on diatomaceous earth or on counter-current distribution is identical to that of prodigiosin. We therefore conclude that a major fraction of the orange pigment of S. marcescens strain OF has been converted to prodigiosin by methylation with diazomethane, and must have structure II. All our evidence from chromatography and counter-current distribution of the crude pigment from this strain points to the complete absence of prodigiosin prior to methylation.

Mild extraction procedures yield more than one pigment fraction from both strain OF (Burgus, 1960) and the wild type (Williams, Green, and Rappoport, 1956; Castro, et al., 1959); therefore it was of interest to see how the concentration of norprodigiosin in strain OF compared with that of prodigiosin in strain Nima. Pigment was extracted from the two strains by identical procedures (except that the extract from OF was treated with diazomethane at the chloroform stage) and chromatographed on alumina; the red prodigiosin band in each case was converted to the perchlorate, which was reconverted to the free base and chromatographed on diatomaceous earth. From 125 g of cells, approximately 230 mg of prodigiosin as the purified free base was isolated in each case.

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