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A NOVEL DIPYRROLYLDIPYRROMETHENE PRODIGIOSIN ANALOG FROM SERRATIA MARCESCENS

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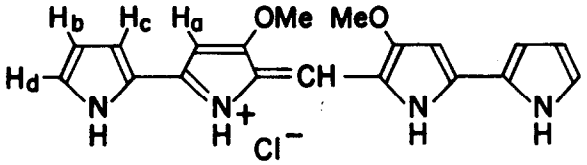
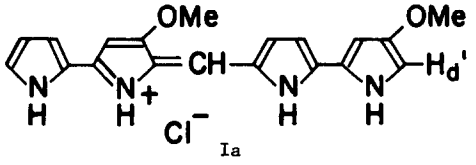
We wish to report the isolation and proof of structure of a new (blue) pigment from a mutant strain of Serratia marcescens. This compound, formulated as the hydrochloride I, represents the first pigment in the prodigiosin series containing two bipyrrole residues.[2,3,4]

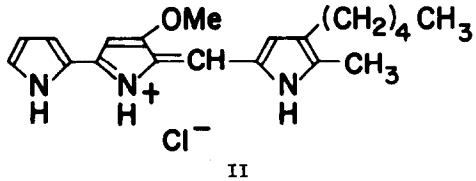


I

Serratia marcescens, XII-114,[5] was grown on a 2% agar gel containing 0.5% Bacto-Peptone and 1.0% glycerol. Eight-day cultures were scraped from the agar, frozen and lyophilized. The dry powder was extracted with methylene chloride and the dark blue solution washed with 1 N NaOH, then 1 N HCl, and dried. After purification by chromatography on acid-washed alumina and recrystallization from CCl_4 the pigment was obtained as the hydrochloride, (blue needles with a green reflex) m.p. 263-265° (dec.). Anal. Calcd. for $C_{19}H_{19}N_4O_2Cl$: C, 61.53; H, 5.16; N, 15.11. Found: C, 61.64; H, 5.22; N, 14.60. The high resolution mass spectrum shows a molecular ion peak at m/e 334 corresponding to the free base, $C_{19}H_{18}N_4O_2$.



Ia

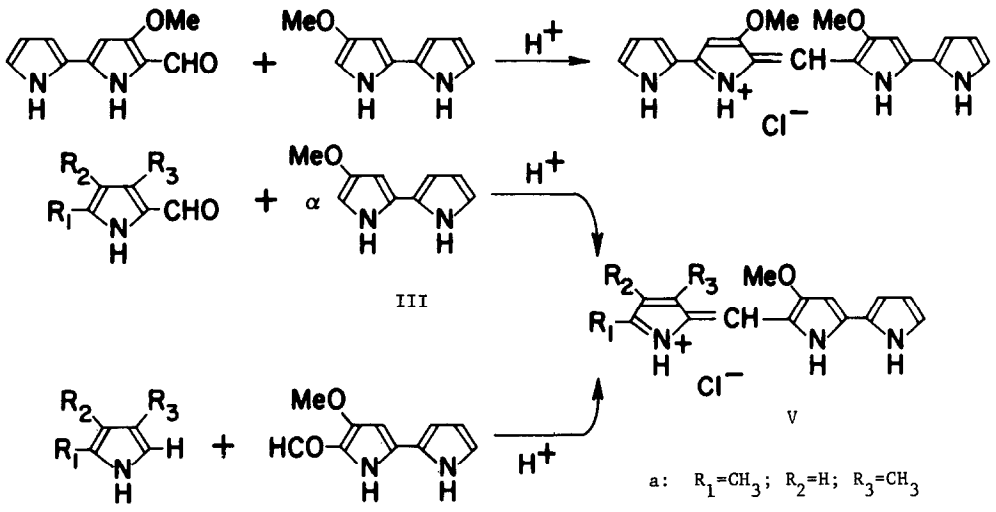


II

Spectroscopic evidence clearly shows that the pigment is a dipyrromethene related to prodigiosin (II). For example, the visible spectrum of the hydrochloride, showing a single peak at 588 mμ with a shoulder at 555 mμ, is strikingly similar to the spectra of prodigiosin salts, and the infrared spectrum has most of the features exhibited by that of II. The appearance of a parent peak at m/e 334 in the mass spectrum strongly suggests that the pigment is made up of two methoxybipyrrole residues as in I or Ia. This was confirmed by the synthesis outlined below.

Condensation of 4-methoxy-2,2'-bipyrrole (III)[6] with 4-methoxy-2,2'-bipyrrole-5-carboxaldehyde (IV)[7] in ethanolic HCl readily yielded the blue pigment (90%). The mixture melting point of the natural and synthetic hydrochlorides was undepressed, and the infrared spectra were superimposable. This synthesis thus establishes the structure of the pigment as either the symmetrical dipyrrolyldipyrromethene I or the unsymmetrical alternative Ia.

The NMR spectrum of the blue pigment strongly supports structure I. The peaks (2H) at τ 3.97, 3.67, 3.18 and 2.87 correspond respectively to the protons at Ha, Hb, Hc and Hd in I, and are in excellent agreement with the comparable absorption of the protons in prodigiosin (II) and in other model systems. On the other hand, the presence (in Ia) of a methoxyl group in place of Hb' would lead to a much more complicated spectrum in which Hd' would show a pronounced shielding effect from the adjacent methoxy group.



We have now been able to confirm structure I for the blue pigment by demonstrating that the acid-catalyzed condensation of methoxy bipyrrole (III) with pyrrole aldehydes take place exclusively at the α-position adjacent to $-OCH_3$. Thus, prodigiosin (II or Vc) and its analogs Va and Vb could be synthesized either by the conventional condensation of prodigiosin precursor (IV) with the appropriate alkyl pyrrole[2a] or, alternatively, by the reaction of methoxybipyrrole (III) with the relevant alkyl pyrrole aldehyde. The identity of each pair of products was established by undepressed mixture melting points, and superimposable IR and (in the case of Va and Vb) NMR spectra.

The above prodigiosin syntheses show clearly that the α-position of III, enriched by electron release from the adjacent methoxyl group, must be the site of reaction with the bipyrrole aldehyde, IV. This evidence thus provides a strong basis for assigning the symmetrical tetrapyrrolic structure (I) to the blue pigment.[8]

Isolation of I from a mutant Serratia strain suggests that 4-methoxy-2,2'-bipyrrole (III) may be an intermediate in the biogenesis of the aldehydic prodigiosin precursor (IV). We have noted, however, that under acidic conditions (ethanolic HCl) IV undergoes partial deformylation, producing a blue pigment identical with the natural material (I). It is therefore possible that the methoxybipyrrole incorporated in the blue pigment arises from the decomposition of the precursor, IV, which is formed by a separate pathway not involving III.

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3. Professor W.R.Hearn has privately informed us that he and R.H. Williams have isolated a blue pigment from Serratia marcescens,IX-3-3, which may be identical with I. (R.H. Williams, Ph.D. dissertation, Iowa State University, (1965).

4. These findings were first reported at the May 1967 meeting of the American Society for Microbiology during a Round Table discussion on Prodigiosin.

5. This mutant was first isolated by M. Bunting and L. Butler at Radcliffe College. It has been classified with Serratia marcescens, IX-3-3, as a mutant of class M-1, D.A. Morrison, J. Bact., 91 (4) 1599 (1966).

6. Prepared by the soda-lime distillation of prodigiosin, G.C. Rodgers, Jr., Ph.D. dissertation, Yale University (1965).

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

8. Although dipyrrolyldipyrromethenes have not previously been isolated from natural sources, representatives of this class have been prepared in the laboratory by R. Grigg and A.W. Johnson, J. Chem. Soc., 3315 (1964).