Prodigiosene [5-(2-Pyrryl)-2,2′-dipyrrylmethene] and Some Substituted Prodigiosenes[1]

WALTER R. HEARN, MICHAEL K. ELSON, ROBERT H. WILLIAMS, AND JORGE MEDINA-CASTRO

Department of Biochemistry and Biophysics, Iowa State University, Ames, Iowa 50010

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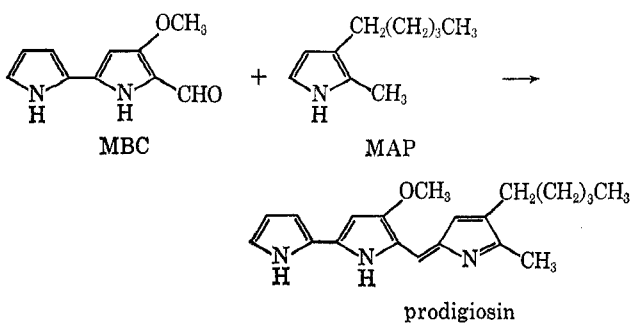
Synthesis of 5-formyl-2,2′-bipyrrole and condensation with pyrrole gave 5-(2-pyrryl)-2,2′-dipyrrylmethene (1). The name prodigiosene is proposed for this compound, parent nucleus of the prodigiosin series of bacterial pigments. Condensation of 5-formyl-2,2′-bipyrrole with substituted pyrroles gave 2-methyl- (3), 2,4-dimethyl- (4), 2,4-dimethyl-3-ethyl- (5), 2-methyl-3-amyl- (6), and 2-(2-pyrryl)prodigiosene (7). Condensation of 2,2′-bipyrrole with 2-formylpyrrole also led to 1, and with 2-acetylpyrrole, to 5-methylprodigiosene (2). Addition of 2,4-dimethyl-3-ethylpyrrole to the growth medium of *Serratia marcescens* strain 9-3-3 gave 2,4-dimethyl-3-ethyl-6-methoxyprodigiosene (8); addition of 2,4-dimethylpyrrole gave 2,4-dimethyl-6-methoxyprodigiosene (9). It is suggested that disproportionation at the methene carbon, as observed with the synthetic compounds, may account for some of the heterogeneity of prodigiosene pigments isolated from natural sources.

The red pyrryldipyrrylmethene pigment characteristic of the microorganism *Serratia marcescens* is called prodigiosin. Since prodigiosin is also a potent but toxic antibiotic,[2] structural analogs are of interest as potentially useful chemotherapeutic agents.[3] Of the prodigiosin-like compounds reported, several are naturally occurring pigments,[4] some are biosynthetic analogs produced by supplying exogenous pyrroles to strains of *S. marcescens*,[5] and others have arisen from synthetic investigations related to prodigiosin[6] or to corrins.[7] Structure of a complex prodigiosin analog isolated from a strain of *Streptomyces longisporus ruber* and containing a *meta*-bridged pyrrole[4g] has recently been confirmed by synthesis.[6d]

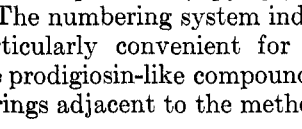
Although many questions about the biosynthesis of prodigiosin remain unanswered,[2] the final step is known to be enzymatic condensation of 5-formyl-4-methoxy-2,2′-bipyrrole (frequently abbreviated MBC, for methoxybipyrrolecarboxaldehyde) with 2-methyl-3-amylpyrrole (frequently abbreviated MAP).[5a]

Acid-catalyzed condensation of the same intermediates led to the first laboratory synthesis of prodigiosin.[6a] An analogous condensation has generally been used for synthesis of prodigiosin-like compounds. A variation is to condense a 2-formylpyrrole with a bipyrrole.[6c] The 2-formylpyrroles and 2-formylbipyrroles are generally obtainable by formylation with dimethylformamide (DMF) and phosphorus oxychloride.[8]



Although perhaps several dozen compounds containing the pyrryldipyrrylmethene nucleus have been described, the unsubstituted parent ring system itself had evidently not been synthesized until our investigation. We obtained it as the hydrobromide salt, both from condensation of 5-formyl-2,2′-bipyrrole with pyrrole and from condensation of 5-formylpyrrole with 2,2′-bipyrrole. Similar reactions led to other structural analogs of prodigiosin. To obtain prodigiosin analogs containing the methoxy group, we also explored the scope of the biosynthetic reaction.

Nomenclature.—We propose the trivial name prodigiosene for the compound 5-(2-pyrryl)-2,2′-dipyrrylmethene (1). The numbering system indicated on the formula is particularly convenient for naming prodigiosin and the prodigiosin-like compounds substituted on the pyrrole rings adjacent to the methene carbon.



prodigiosene (1)

Prodigiosin is thus 2-methyl-3-amyl-6-methoxyprodigiosene, a name that retains the numbering of one of the biological precursors, 2-methyl-3-amylpyrrole.[5a] The overall numbering system also avoids the confusion possible when prime numbers are used by different investigators[4c,7] to designate different rings in the

(1) (a) Journal Paper No. J-6078 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa, Project No. 1384; (b) this investigation was supported in part by Research Grant AI-05016 of the National Institute for Allergy and Infectious Diseases, U. S. Public Health Service; (c) taken in part from the Ph.D. theses of R. H. Williams (1965), M. K. Elson (1968), and J. Medina-Castro (1968), Iowa State University Library, Ames, Iowa.

(2) R. P. Williams and W. R. Hearn in "Antibiotics. Vol. II. Biosynthesis," D. Gottlieb and P. D. Shaw, Ed., Springer-Verlag, Berlin, Germany, 1967, pp 410–432.

(3) A. J. Castro, G. R. Gale, G. E. Means, and G. Tertzakian, *J. Med. Chem.*, **10**, 29 (1967).

(4) (a) W. R. Hearn, R. E. Worthington, R. C. Burgus, and R. P. Williams, *Biochem. Biophys. Res. Commun.*, **17**, 517 (1964); (b) H. H. Wasserman, G. C. Rodgers, Jr., and D. D. Keith, *Chem. Commun.* 825 (1966); (c) K. Harashima, N. Tsuchida, T. Tanaka, and J. Nagatsu, *J. Agr. Biol. Chem.* (Tokyo), **31**, 481 (1967); (d) Y. M. Khokhlova, A. V. Puchnina, and O. I. Artamonova, *Biokhimiya*, **29**, 841 (1964); (e) H. H. Wasserman, D. J. Friedland, and D. A. Morrison, *Tetrahedron Lett.*, 641 (1968); (f) M. S. Feider, unpublished M.S. thesis, Iowa State University Library, Ames, Iowa, 1968; (g) H. H. Wasserman, G. C. Rodgers, and D. D. Keith, *J. Amer. Chem. Soc.*, **91**, 1263 (1969).

(5) (a) H. H. Wasserman, J. E. McKeon, and U. V. Santer, *Biochem. Biophys. Res. Commun.*, **3**, 146 (1960); (b) H. H. Wasserman, J. E. McKeon, L. A. Smith, and P. Forgione, *Tetrahedron, Suppl.*, **8**, part II, 647 (1966); (c) R. P. Williams, M. E. Goldschmidt, and C. L. Gott, *Biochem. Biophys. Res. Commun.*, **19**, 177 (1965).

(6) H. Rapoport and K. G. Holden, *J. Amer. Chem. Soc.*, **84**, 635 (1962); (b) H. H. Wasserman, J. Keggi, F. Bohlman, and W. Luders, *Angew. Chem.*, **72**, 779 (1960); (c) A. Ermili and A. J. Castro, *J. Heterocycl. Chem.*, **3**, 521 (1966); (d) H. H. Wasserman, D. D. Keith, and J. Nadelson, *J. Amer. Chem. Soc.*, **91**, 1264 (1969).

(7) E. Bullock, R. Grigg, A. W. Johnson, and J. W. F. Wasley, *J. Chem. Soc.*, 2326 (1963).

(8) R. M. Silverstein, E. E. Ryskiewicz, and C. Willard, *Org. Syn.*, **36**, 74 (1956).

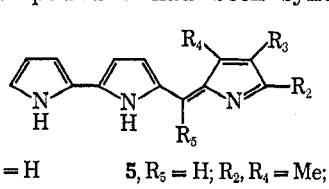
molecule. In our system, the compound designated by others[7] ethyl 5-(3'',4''-dimethylpyrrol-2''-yl)-3,3',4,5'-tetramethyldipyrromethene-4'-carboxylate becomes ethyl 2,4,6,7,8,9-hexamethylprodigiosene-3-carboxylate, which we consider easier to visualize.

The name prodigiosene ultimately derives from Kraft,[9] who extracted red "prodigiosine" from *Bacillus prodigiosus*. The rod-shaped organism (also known as *Bacterium prodigiosum*) is now preferably designated *Serratia marcescens*.[10] Trivial names given to partially characterized natural products from *S. marcescens* include serratin, a pigment evidently related to prodigiosin,[11] and marcescin, an antibiotic evidently of peptide nature.[12] A group of Russian investigators[13] who use the older nomenclature for the organism have given the name prodigiosan to a heteropolysaccharide isolated from it and possessing antitumor activity. The antitumor properties of polysaccharide–peptide complexes extracted from *S. marcescens* have also been studied by others.[14] We suggest that the name prodigiosane be reserved for the pyrryldipyrrylmethane structure hypothetically derivable by reduction of prodigiosene.

Prodigiosene retains the "-ene" suffix of pyrryldipyrrylmethene to suggest its completely conjugated structure. Although clearly distinguishable from prodigiosin, the name for the parent nucleus was deliberately chosen to be as similar as possible to the name of its best-known naturally occurring derivative. During preparation of this manuscript we learned of an alternative nomenclature proposed by Dr. Nancy N. Gerber of the Institute of Microbiology of Rutgers University.[15] She chose the name prodiginine for what we term 6-methoxyprodigiosene, having isolated a naturally occurring nonyl derivative of it and wishing to avoid the ambiguity of "nonylprodigiosin." However, we feel that our more comprehensive system of nomenclature is preferable because of its convenient applicability to synthetic compounds as well as to the naturally occurring species of pigment.

Results and Discussion

Compounds **1–4**, **6**, and **7** are new prodigiosene pigments. Compound **5** had been synthesized previously by a different route[6c] and characterized as the hydrobromide, perchlorate, and free base, but no spectral data were reported. Compound **9** had been obtained biosynthetically[5a] and later characterized as the perchlorate.[5b] Compound **8** had been prepared biosynthetically[5c] but not characterized.



1, $R_2, R_3, R_4, R_5 = H$
2, $R_2, R_3, R_4 = H$; $R_5 = Me$
3, $R_3, R_4, R_5 = H$; $R_2 = Me$
4, $R_3, R_5 = H$; $R_2, R_4 = Me$
5, $R_5 = H$; $R_2, R_4 = Me$; $R_3 = Et$
6, $R_4, R_5 = H$; $R_2 = Me$; $R_3 = n$-amyl
7, $R_3, R_4, R_5 = H$; $R_2 =$ 2-pyrryl

Ermili and Castro[6c] attempted the HBr-catalyzed condensation of 2,2'-bipyrrole with a number of 2-formylpyrroles. Initial color changes of their reaction mixtures indicated formation of prodigiosenes, but they were able to isolate as products only **5** and 3,5-dimethyl-4-ethoxycarbonylprodigiosene. In particular, they were unable to isolate **1** from condensation of bipyrrole with 2-formylpyrrole itself. We had better luck and also found that bipyrrole would condense under slightly more vigorous conditions (refluxing ethanol under nitrogen) with 2-acetylpyrrole to give **2**.

Except for the 6-methoxy group, or a 6-hydroxy group,[4a] substituents on all known naturally occurring prodigiosenes are limited to the monopyrrole moiety. It was therefore of more general interest to synthesize prodigiosene derivatives *via* 5-formyl-2,2'-bipyrrole. This compound had evidently not been prepared before, although highly substituted derivatives are known[7] in addition to the bipyrrole prodigiosin precursor MBC and its 3-methoxy isomer.[6a] Formylation of 2,2'-bipyrrole in DMF as solvent gave a 60% yield of the desired monoformyl compound. Condensation of formylbipyrrole with pyrrole itself gave **1**, and with other pyrroles, compounds **3–7**. Compound **6** is demethoxyprodigiosin, of interest for comparative studies of biological activity.

For preparation of 6-methoxyprodigiosenes we utilized shake cultures of *S. marcescens* strain 9-3-3 as a source both of MBC and of the final "prodigiosin synthetase" enzyme.[16] Added 2,4-dimethyl-3-ethylpyrrole ("cryptopyrrole") was readily used by the normally nonpigmented organism as a substitute for MAP and **8** was obtained as the crystalline perchlorate. Utilization of added 2,4-dimethylpyrrole was less efficient and purification of **9** was difficult because of a persistent contaminant showing aliphatic protons in nmr spectrometry, although Wasserman[5b] reported no difficulties in purifying **9** after exposing surface cultures of the same strain to vapors of 2,4-dimethylpyrrole. In our system, 2-methylpyrrole was barely utilized for prodigiosene synthesis, and pyrrole itself, indole, 2-formylpyrrole, and 2,5-dimethylpyrrole were not utilized at all. These results indicate a low order of enzyme specificity for the monopyrrole moiety, provided that the pyrrole has a free α position and sufficient alkyl substitution.

The fact that one *Serratia* mutant (strain OF), unable to methylate 5-formyl-4-hydroxy-2,2'-bipyrrole,[17] converts it to norprodigiosin[4a] may indicate some breadth in specificity for the bipyrrole moiety also. It was therefore of interest to test 5-formyl-2,2'-bipyrrole as a potential substrate in another mutant which produced the monopyrrole portion of prodigiosin and also contained active synthetase enzyme. Addition of formylbipyrrole to 24-hr shake cultures of *S. marcescens* strain WF[18] failed to stimulate pigment production within 12 hr, although presence of the enzyme and

(9) E. Kraft, unpublished thesis, Würzburg, Germany, 1902; cited by F. Wrede and O. Hettche, *Ber.*, **62**, 2678 (1929).
(10) R. S. Breed and M. E. Breed, *J. Bacteriol.*, **9**, 545 (1924).
(11) A. Treibs and R. Zimmer-Galler, *Z. Physiol. Chem.*, **318**, 12 (1960).
(12) A. T. Fuller and J. M. Horton, *J. Gen. Microbiol.*, **4**, 417 (1950).
(13) Z. V. Ermol'eva, D. M. Trakhtenberg, and B. N. Bondarenko, *Antibiotiki*, **5**, 397 (1964).
(14) (a) H. J. Creech, E. R. Brueniger, and G. A. Adams, *Can. J. Biochem.*, **42**, 593 (1964); (b) S. M. Navashin, I. P. Fomina, and T. G. Terent'eva, *Antibiotiki*, **10**, 1011 (1965).
(15) N. N. Gerber, *Applied Microbiol.*, in press.
(16) P. P. Mukherjee, M. E. Goldschmidt, and R. P. Williams, *Biochim. Biophys. Acta*, **136**, 182 (1967).
(17) W. R. Hearn, R. E. Worthington, R. C. Burgus, and L. Bascur de Medina, *Federation Proc.*, **22**, 354 (1963).
(18) R. P. Williams and J. A. Green, *J. Bacteriol.*, **72**, 537 (1956).

monopyrrole could be demonstrated by addition of MBC.

Spectra.—Analytical and spectral data for the compounds prepared in this investigation were consistent with the expected structures.[19] The spectrophotometric titration data may be compared with pK_a values reported for prodigiosin (8.25)[20] and "undecylprodigiosin" (7.62).[4c]

Mass spectra of **8** and **9** were obtained on the hydrochlorides and those of **1–7** on the hydrobromides. Thermal decomposition of the salts within the ion source[21] gave mass spectra of the free bases. Major peaks in the mass spectra could be explained on the basis of published spectra of related compounds. Prodigiosene, for example, showed a strong molecular ion (*m/e* 209) and no other significant peaks to *m/e* 132, base peak of the spectrum. Peaks at *m/e* 105 and 104 were also present. The spectrum of 2,2′-bipyrrole reported by Rodgers[22] had the molecular ion (*m/e* 132) as its base peak, accompanied by M − 27 (*m/e* 105) and M − 28 (*m/e* 104). These peaks are in accord with a fragmentation mechanism proposed for pyrrole.[23] The spectrum of **1** also had a peak (*m/e* 80) characteristic of methylpyrrole and, finally, at *m/e* 66 a peak indicating fragmentation at either the pyrryl–pyrrole bond or the pyrryl–methene bond.

It should be noted that the peak at *m/e* 132 (base peak of the spectrum of **1** and prominent in spectra of **2–7**) was not the peak (*m/e* 131) expected from ion-induced fragmentation of the bond between the bipyrrole and the methene carbon. Cleavage of these methene bonds was probably the result of pyrolysis from the heating necessary to decompose the hydrobromide salt and volatilize the molecule in the mass spectrometer before ionization. This possibility is supported by the fact that pyrolysis of naturally occurring prodigiosene pigments has been a fruitful degradative method in several studies.[22,24] In our laboratory the monopyrrole fragment has been the only isolable product, but in Wasserman's laboratory[22] bipyrroles as well as monopyrroles have been isolated after pyrolysis. Some fragmentation of pyrrylmethene bonds in the mass spectrometer was observed by Jackson, *et al.*,[25] in the mass spectra of more than a dozen dipyrrylmethene hydrobromides, but ions representing fragmentation of the methene bonds did not exceed 17% of the base peaks. With the prodigiosene derivatives described here, ions with *m/e* of 1 greater than expected from ionization-induced fragmentation of the methene bonds were found, and in much greater abundance than expected from the dipyrrylmethene analogies.

(19) Complete ultraviolet–visible, ir, nmr, and mass spectra of 5-formyl-2,2′-bipyrrole and compounds **1–7** and mass spectra of **8** and **9** are recorded in M. K. Elson's unpublished Ph.D. thesis, Iowa State University Library, Ames, Iowa, 1968. Electronic, ir, and nmr spectra of **8** and **9** are recorded in R. H. Williams' unpublished Ph.D. thesis, Iowa State University Library, Ames, Iowa, 1965.

(20) W. R. Hearn, J. Medina-Castro, and M. K. Elson, *Nature*, **220**, 170 (1968).

(21) K. Biemann and J. A. McCloskey, *J. Amer. Chem. Soc.*, **84**, 3192 (1962).

(22) G. C. Rodgers, Jr., Unpublished Ph.D. thesis, Yale University Library, New Haven, Conn., 1965.

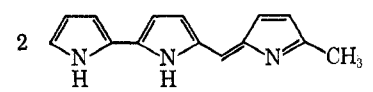
(23) H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Interpretation of Mass Spectra of Organic Compounds," Holden-Day Co., San Francisco, Calif., 1964, pp 237–239.

(24) F. Wrede and A. Rothhaas, *Z. Physiol. Chem.*, **219**, 267 (1933).

(25) A. H. Jackson, G. W. Kenner, H. Budzikiewicz, C. Djerassi, and J. M. Wilson, *Tetrahedron*, **23**, 603 (1967).

Mass spectra of the 6-methoxyprodigiosenes **8** and **9** were similar to the spectrum of prodigiosin published by Jackson, *et al.*[25] Unlike prodigiosin, however, both **8** and **9** exhibited an M − 31 ion, with a corresponding metastable peak.

Disproportionation Reactions.—During the synthesis of compounds **1–6**, a small amount of 2-(2-pyrryl)-prodigiosene (**7**) was observed as a by-product in each instance. Its identity was shown by comparison with an authentic sample on tlc and by the appearance of a small peak (*m/e* 274) in the mass spectra. Compound **7** could have been produced by deformylation and condensation of the resulting 2,2′-bipyrrole with formylbipyrrole.[4e] Such a reaction has been reported for formylpyrroles.[26] Deformylation may account for the presence of **7** in the original reaction mixtures, but **7** was also produced when purified prodigiosene derivatives were allowed to stand in solution. The rate of formation in ethanol was much greater than in chloroform. In *ca.* 2 hr an ethanolic solution of **3** was converted in 80% yield to **7**. Chromatography of various prodigiosene hydrobromides stored for several weeks in the dark as crystals showed the presence of some **7**.



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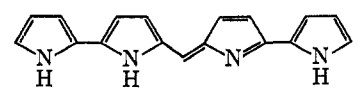

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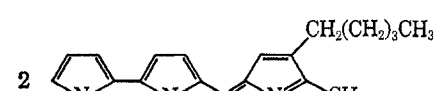
Prodigiosene derivatives with highly substituted monopyrrole rings showed a tendency to disproportionate to dipyrrylmethenes as well. In the mass spectrum of **9**, ions with *m/e* ratios expected for a tetramethyldipyrrylmethene were observed. To a lesser extent, analogous dipyrrylmethene ions were observed with compounds **4**, **5**, and **6**. The abundance of these ions increased with the temperature and time of the sample in the mass spectrometer.



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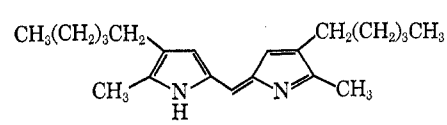

When 6-methoxyprodigiosenes are being purified from natural sources, traces of additional pigments are almost invariably encountered, even late in the purification process. The type of pyrryl–bipyrryl exchange behavior observed here may offer an explanation for the

(26) A. J. Castro, G. Tertzakian, B. T. Nakata, and D. A. Brose, *ibid.*, **33**, 4499 (1967).

presence of some of these pigments, particularly of those which appear to be artifacts.

Experimental Section[27]

Starting Materials.—Pyrrole, 2-formylpyrrole, 2,5-dimethylpyrrole, and 2,4-dimethyl-3-ethylpyrrole were obtained from Aldrich Chemical Co., Milwaukee, Wis.; 2,4-dimethylpyrrole from K & K Laboratories, Plainview, N. Y.; and indole from Distillation Products, Inc., Rochester, N. Y. The method of Oddo and Dainotti[28] was used to prepare 2-acetylpyrrole; recrystallized from water, it had a melting point of 90°. 2-Methylpyrrole and 2-methyl-3-amylpyrrole were prepared according to Castro, *et al.*,[29] from 2-formylpyrrole and 2-methyl-3-valeroyl-3-ethoxycarbonylpyrrole. After fractional distillation, the 2-methylpyrrole, bp 148°, showed a single peak on vpc; the 2-methyl-3-amylpyrrole was used directly without distillation. 2,2′-Bipyrrole was prepared essentially according to Rapoport and Castagnoli[30] by hydrogenation of 2,2′-(1-pyrrolinyl)pyrrole over 10% palladium on charcoal; after sublimation and recrystallization from benzene, it had a melting point of 189–190°.

5-Formyl-2,2′-bipyrrole.—The technique used by Bullock, *et al.*,[7] for preparing highly substituted formylbipyrroles was successful for the unsubstituted compound. One gram (7.5 mmol) of 2,2′-bipyrrole was dissolved in 10 ml of DMF in a flask fitted with stirrer, nitrogen sweep, and dropping funnel and cooled in an ice bath. A complex of 1.16 g (7.5 mmol) of $POCl_3$ and 1 ml (13 mmol) of DMF was added dropwise over a 15-min period. The reaction mixture turned green on addition of the complex and then solidified. The ice bath was removed and the reaction mixture was allowed to stand at room temperature for 30 min. The contents of the flask were dissolved in water, and 10% NaOH was added until the odor of dimethylamine was detected. The mixture was then warmed slightly, occasionally with addition of more NaOH, and the product crystallized. The precipitate was collected by filtration, dissolved in EtOH, decolorized with charcoal, and crystallized from EtOH–water to give 0.74 g (61%) of formylbipyrrole: mp 234–240° dec; uv max (MeOH) 360 (ε 27,500) and 236 nm (ε 12,400); nmr (DMSO-d_6) δ 6.14 (m, 1), 6.50 (d, 1, J = 4 Hz), 6.72 (m, 1), 6.90 (m, 1), 7.00 (d, 1, J = 4 Hz), 9.37 (s, 1, HCO), 11.21 (br, 1, NH), and 11.97 (br, 1, NH); mass spectrum (70 eV) m/e (rel intensity) 160 (100), 131 (59), 105 (5), and 104 (25).

Anal. Calcd for $C_9H_8N_2O$: C, 67.5; H, 5.04; N, 17.5. Found: C, 67.50; H, 4.87; N, 17.72.

Hydrobromide of Prodigiosene (1). Procedure A.—A solution of 132 mg (1 mmol) of 2,2′-bipyrrole and 95 ml (1 mmol) of 2-formylpyrrole in 5 ml of EtOH was warmed on a steam bath. On addition of 7 drops of 48% HBr, the solution immediately turned deep purple. The crystals that formed in about 30 min were collected by filtration and dissolved in warm $CHCl_3$. The warm solution was filtered and an equal volume of ligroin (Skellysolve B) was added dropwise while the solution was cooling. Long metallic-blue needles were collected and vacuum dried at 40° to give 62 mg (21%) of the hydrobromide of **1**: mp 360°; visible pK_a 7.20, max (95% EtOH, HCl) 541 nm (ε 64,000), (95% EtOH, NaOH) 455 nm (ε 32,700); nmr ($CDCl_3$) δ 6.45 (m, 2), 6.8–7.4 (complex m, 6),7.58 (m, 1), and 9.33 (br, 2, NH); mass spectrum (70 eV) m/e (rel intensity) 209 (9), 132 (100), 131 (41), 105 (20), 104 (35), 82 (37), 80 (39), and 66 (18).

Anal. Calcd for $C_{18}H_{12}N_3Br$: C, 53.8; H, 4.14; N, 14.5. Found: C, 53.71; H, 4.20; N, 14.44.

Procedure B.—The hydrobromide of **1**, identical with the preceding compound by tlc and spectral properties, was prepared in a similar manner from 5-formyl-2,2′-bipyrrole and redistilled pyrrole.

Hydrobromide of 5-Methylprodigiosene (2).—An EtOH solution of 132 mg (1 mmol) of 2,2′-bipyrrole and 109 mg (1 mmol) of 2-acetylpyrrole was brought to reflux under nitrogen. On addition of 7 drops of 48% HBr the solution turned purple and slowly darkened during 2 hr of refluxing. Evaporation of the EtOH left a residue which was chromatographed on silicic acid developed with 15% acetone–$CHCl_3$. The major band was crystallized from $CHCl_3$–ligroin to yield 39 mg (13%) of the hydrobromide of **2**: mp 208–209°; visible pK_a 6.65, max (95% EtOH, HCl) 550 nm (ε 62,500), (95% EtOH, NaOH) 461 nm (ε 28,400); nmr ($CDCl_3$, obtained at 100 MHz and chemical shifts converted to 60 MHz) δ 2.72 (d, 3, J = 7 Hz, CH_3) and 6.18–7.7 (complex m, 8); mass spectrum (70 eV) m/e (rel intensity) 223 (44), 208 (12), 132 (100), 105 (27), and 104 (35).

Anal. Calcd for $C_{14}H_{14}N_3Br$: C, 55.3; H, 4.61; N, 13.8. Found: C, 55.44; H, 4.70; N, 13.76.

General Procedure for Compounds 3–7.—As in procedure B described for **1**, 160 mg (1 mmol) of 5-formylbipyrrole and 1 mmol of the appropriate pyrrole were allowed to react. The hydrobromides were all crystallized from $CHCl_3$–ligroin.

Hydrobromide of 2-Methylprodigiosene (3).—From 81 mg of 2-methylpyrrole was obtained 42 mg (14%) of the purple hydrobromide of **3**, plus a smaller second crop: mp 179–180° dec; visible pK_a 8.10, max (95% EtOH, HCl) 553 nm (ε 80,200), (95% ETOH, NaOH) 470 nm (ε 36,100); nmr ($CDCl_3$) δ 2.63 (s, 3, CH_3), 6.10–7.28 (complex m, 8), and 9.50 (br, 2, NH); mass spectrum (70 eV) m/e (rel intensity) 223 (28), 208 (6), 132 (18), 105 (9), 104 (12), 81 (60), 80 (100), and 79 (50).

Anal. Calcd for $C_{14}H_{14}N_3Br$: C, 55.3; H, 4.61; N, 13.8. Found: C, 55.44; H, 4.70; N, 13.76.

Hydrobromide of 2,4-Dimethylprodigiosene (4).—From 95 mg of 2,4-dimethylpyrrole was obtained 116 mg (36%) of long blue needles of the hydrobromide of **4**: mp 203–204.5°; visible pK_a 8.23, max (95% EtOH, HCl) 553 nm (ε 96,600), (95% EtOH, NaOH) 482 nm (ε 43,100); nmr ($CDCl_3$) δ 2.28 (s, 3, CH_3), 2.64 (s, 3, CH_3), 6.05–7.25 (complex m, 6), and 9.07 (br, 2, NH); mass spectrum (70 eV) m/e (rel intensity) 237 (95), 236 (64), 222 (12), 207 (7), 132 (19), and 94 (100).

Anal. Calcd for $C_{15}H_{16}N_3Br$: C, 56.6; H, 5.04; N, 13.2. Found: C, 56.67; H, 5.10; N, 13.34.

Hydrobromide of 2,4-Dimethyl-3-ethylprodigiosene (5).—From 123 mg of 2,4-dimethyl-3-ethylpyrrole was obtained 164 mg (48%) of green crystals of the hydrobromide of **5**: mp 229.5–231° (lit.[6c] mp 232–233° dec); visible pK_a 8.40, max (95% EtOH, HCl) 559 nm (ε 95,700) (95% EtOH, NaOH) 492 nm (ε 39,800); nmr ($CDCl_3$) δ 1.06 (t, 3, J = 7.5 Hz, CH_2CH_3), 2.18 (s, 3, CH_3), 2.38 (q, 2, J = 7.5 Hz, CH_2CH_3), 2.62 (s, 3, CH_3), 6.25–7.24 (complex, 6), and 9.63 (br, 2, NH); mass spectrum (70 eV) m/e (rel intensity) 265 (100), 264 (41), 250 (25), 235 (13), and 132 (12).

Anal. Calcd for $C_{17}H_{20}N_3Br$: C, 58.9; H, 5.78; N, 12.1. Found: C, 58.94; H, 5.84; N, 12.03.

Hydrobromide of 2-Methyl-3-amylprodigiosene (6).—Crude 2-methyl-3-amylpyrrole from reduction of 1.0 g of 2-methyl-3-valeroyl-5-ethoxycarbonylpyrrole[29] gave a purple solid that was chromatographed on silicic acid. Elution with 10% acetone–$CHCl_3$ brought off a major band followed by a fraction containing **3**. The major fraction was crystallized from $CHCl_3$–ligroin to give tiny, dark purple crystals of the hydrobromide of **6**: yield 59 mg; mp 123–123.5°; visible pK_a 8.35, max (95% EtOH, HCl) 562 nm (ε 95,500), (95% EtOH, NaOH) 485 nm (ε 39.500); nmr ($CDCl_3$) δ 0.91 (m, 3, CH_2CH_3), 1.37 (m, 6 CH_2), 2.37 (m, 2, CH_2 attached to ring), 2.62 (s, 3, CH_3), 6.20–7.25 (complex, 7), and 9.0 (br, 2, NH); mass spectrum (70 eV) m/e (rel intensity) 293 (47), 278 (8), 236 (34), 165 (50), 151 (42), 132 (13), 94 (100), and 80 (46).

Anal. Calcd for $C_{19}H_{24}N_3Br$: C, 60.7; H, 6.45; N, 11.4. Found: C, 60.86; H, 6.54; H, 11.24.

Hydrobromide of 2-(2-Pyrryl)prodigiosene (7).—From 132 mg of 2,2′-bipyrrole was obtained 222 mg (63%) of blue crystals of

(27) Melting points, uncorrected, were obtained with a Mel-Temp capillary apparatus. Evaporation of solvents was accomplished with a Büchi Rotavapor with bath temperature below 35°. Elemental analyses were performed by Ilse Beetz Mikroanalytisches Laboratorium, 8640 Kronach, Postfach 460, West Germany. Electronic absorption spectra were obtained with a Cary Model 15 or occasionally a Beckman DU spectrophotometer; ir spectra (KBr) were obtained with a Perkin-Elmer Model 21 spectrometer (see ref 19); and nmr spectra were obtained with a Varian Model A-60 spectrometer, using $SiMe_4$ as internal standard. Mass spectra were obtained with an Atlas CH-4 mass spectrometer equipped with a TO-4 ion source. The pH measurements were made with a Beckman Model 76 expanded-scale pH meter. Apparent pK_a values were obtained from plots of absorbance at λ_{max} for both acid and basic forms; 5 ml of a 95% EtOH solution of the prodigiosene was made up to 10 ml in a volumetric flask with one of the four buffers of ref 20, covering the range of pH 6–11. Synthetic reactions and purification procedures were monitored by tlc on silica gel G (E. Merck) and colorless pyrroles were visualized with an Ehrlich spray reagent of *p*-dimethylaminobenzaldehyde in MeOH–HCl.

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the hydrobromide of **7**: mp 215° dec; visible pK_a 7.90, max (95% EtOH, HCl) 649 nm (ϵ 106,000), (95% EtOH, NaOH) 545 nm (ϵ 35,200); nmr ($CDCl_3$) δ 6.2–7.23 (complex, 7) and 9.40 (m, 3, NH); mass spectrum (70 eV) *m/e* (rel intensity) 274 (5), 132 (100), 105 (46), and 104 (60).

Anal. Calcd for $C_{17}H_{15}N_4Br$: C, 57.5; H, 4.26; N, 15.8. Found: C, 57.34; H. 4.79; N, 15.29.

Biosynthetic Experiments.—All cultures were grown at 28° in the dark. *Serratia marcescens* strains 9-3-3 and WF were cultured for 24 hr in the complex medium of Williams, *et al.*,[31] and the cultures were preserved in small screw-cap vials at deep-freeze temperature. Accumulation of 5-formyl-4-methoxy-2,2′-bipyrrole (MBC) in strain 9-3-3 grown on the 0.5% peptone–1.0% glycerol medium of Bunting, *et al.*,[32] was monitored spectrophotometrically[33] (A_{363}–A_{400}) and found to approach a maximum by 48 hr of vigorously shaken culture. Pigmentation (A_{537}–A_{655}) in this strain was negligible at 48 hr in control flasks and in those to which pyrrole, indole, 2-formylpyrrole, or 2,5-dimethylpyrrole had been added at 24 hr. After addition of 2-methylpyrrole at 24 hr, both cells and medium were slightly orange by 48 hr, but pooled ligroin extracts of 5 l. of total culture contained too small an amount of pigment to warrant further investigation.

A 5-ml portion of a 24-hr culture of strain WF was inoculated into each of three 2-l. flasks containing 400 ml of autoclaved Williams' broth. The flasks were lightly stoppered with sterile cotton and shaken for 24 hr. After addition to each flask of 50 mg of 5-formyl-2,2′-bipyrrole dissolved in EtOH, the flasks were returned to the shaker. After 2 hr no pigment had been produced. However, when a few milligrams of MBC was added to one flask and the flask was returned to the shaker, a pink color was evident within 15 min. After 6 hr a second flask gave the same results. At 12 hr the third flask was removed; no pigment was evident in the cells; and no pigment was produced by acidification of the slightly greenish culture filtrate with 1 ml of concentrated HCl.

2,4-Dimethyl-3-ethyl-6-methoxyprodigiosene (8).—Twelve 2-l. erlenmeyer flasks each containing 200 ml of peptone–glycerol broth were autoclaved and the pH was adjusted to 7.5. Each flask was inoculated with 2 ml of a 24-hr inoculum of *S. marcescens* strain 9-3-3, stoppered lightly with sterile cotton, and shaken in a gyratory shaker–incubator. After 24 hr, 0.4 ml of a solution containing 50 mg/ml of 2,4-dimethyl-3-ethylpyrrole was pipetted into each flask. The cultures began to turn red almost immediately, and, after another 24 hr of shaking, resembled 48-hr cultures of wild-type *Serratia* strain Nima. The cells were harvested by centrifugation and treated with aqueous NaOH (turning them from red to brown). The pigment was extracted into ligroin, precipitated as the hydrochloride, and converted to the perchlorate, using procedures developed by Wrede[34] for prodigiosin from wild-type strains. For chromatography on diatomaceous earth (Hy-flo Super-cel, Johns–Manville Co.) the perchlorate was converted to the free base and the column was developed with 0.2% MeOH–ligroin. The major red-orange fraction was taken to dryness, redissolved in warm 95% EtOH, and treated with 5% aqueous $HClO_4$ dropwise. An average of 90–100 mg of crystalline perchlorate of **8** was obtained from 2.4 l. of culture. The **free base** had nmr ($CDCl_3$) δ 0.93 (t, 3, CH_2-CH_3), 1.25 (s, 2, contaminant), 1.73 (s, 3, CH_3), 2.09 (s, 3, CH_3) superimposed on 2.23 (q, 2, CH_2CH_3), 3.91 (s, 3, OCH_3), and 6.05–6.90 (complex, 5). The **hydrochloride** had mass spectrum (70 eV) *m/e* (rel intensity) 295 (100), 280 (29), 264 (25), 249 (5), and 235 (12). The **perchlorate** had visible max (95% EtOH, HCl) 537 nm (ϵ 122,000), (95% EtOH, NaOH) 465 nm (ϵ 51,200).

Anal. Calcd for $C_{18}H_{21}N_3O \cdot HClO_4$: C, 54.6; H, 5.57; N, 10.6. Found: C, 55.2; H, 5.82; N, 10.2.

2,4-Dimethyl-6-methoxyprodigiosene (9).—In a manner similar to that used to obtain **8**, addition of 2,4-dimethylpyrrole at 24 hr to cultures of *S. marcescens* strain 9-3-3 gave **9** in amounts of about 20–25 mg/2.4 l. of culture. The **perchlorate** decomposed above 250° (lit.[5b] mp *ca.* 260° dec): visible max (95% EtOH, HCl), 527 nm (ϵ 110,000), (95% EtOH, NaOH) 465 nm (ϵ 44,000) (lit.[5b] same). The **free base** had nmr ($CDCl_3$) δ 0.87–1.60 (contaminant), 1.82 (s, 3, CH_3), 2.14 (s, 3, CH_3), 3.91 (s, 3, OCH_3), 5.66–6.88 [complex, 6, includes 5.66 (s, 1, ring H on monopyrrole β position)].

Anal. Calcd for $C_{16}H_{17}N_3O$: C, 71.9; H, 6.37; N, 15.7. Found: C, 71.3; H, 7.85; N, 11.1.

The **hydrochloride** had mass spectrum (70 eV) *m/e* (rel intensity) 267 (100), 252 (24), 236 (39), and 221 (5).

Registry No.—**1**, 22187-69-5; **2** hydrobromide, 22187-70-8; **3** hydrobromide, 22187-72-0; **4** hydrobromide, 22187-73-1; **5** hydrobromide, 13672-01-0; **6** hydrobromide, 22187-75-3; **7** hydrobromide, 22232-82-2; **8**, 22187-76-4; **8** perchlorate, 22187-86-6; **9**, 2030-62-8; 5-formyl-2,2′-bipyrrole, 22187-87-7.

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