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THE STRUCTURE OF METACYCLOPRODIGIOSIN

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Abstract—Metacycloprodigiosin, an analog of prodigiosin, was isolated from *Streptomyces longisporus ruber*, and shown to have structure **1**. An exhaustive reduction technique, instrumental in elucidating the structure of **1** has been generally applied to the reduction of alkylpyrroles. Novel features in the mass spectral cracking pattern of alkylpyrroles and other heterocyclic systems are discussed.

In an accompanying paper[1] we reported on the structure and synthesis of undecylprodigiosin **2**, a C-25 prodigiosin analog isolated from *Streptomyces longisporus ruber*. It was noted that another more complex C-25 prodigiosin-like pigment† is formed concurrently with **2**. This substance, which we have named metacycloprodigiosin,‡ has been described in our preliminary communication.[3] Based on spectroscopic, degradative, and synthetic studies we have assigned structure **1** to this compound. As such, this new tripyrrole pigment incorporates the unusual structural feature of a *meta*-bridged pyrrole, the first such system to be observed in a natural product. We are now reporting the full details of our investigations on the isolation and structure determination of this new prodigiosin analog, and in the following paper,[4] we record the total synthesis of the racemic modification of the pigment.

†There have been numerous reports of C-25 analogs of prodigiosin of undetermined structure.[2] A number of these substances, some of which show considerable antibiotic activity, most probably correspond to **1** or **2**.

‡For proposals on systematic nomenclature in the prodigiosin series see: N. N. Gerber, *Appl. Microbiol.* **18**, 1 (1969); W. R. Hearn, M. K. Elson, R. H. Williams and J. Medina-Castro, *J. Org. Chem.* **35**, 142 (1970).

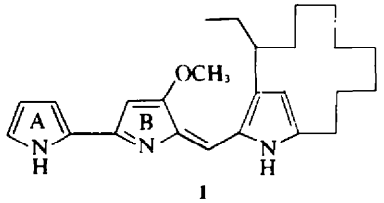
§The strains of *Streptomyces longisporus ruber*, strain M-3, were kindly provided by Dr. K. Haider, Institut fur Biochemie des Bodens, Braunschweig, Germany.

Isolation and spectroscopic studies

Streptomyces longisporus ruber, strain M-3,§ was grown on a soymeal-mannitol medium in shake culture for 1–3 weeks. Methylene chloride extraction of the lyophilized cells followed by acid and base washing and removal of the solvent *in vacuo* yielded a dark amorphous solid. Chromatography of the solid on basic alumina yielded two fractions, the first containing metacycloprodigiosin **1** and the second containing a mixture of **1** and undecylprodigiosin **2**.[1] Metacycloprodigiosin **1** was obtained as orange-brown crystals from petroleum ether, m.p. 208–210°, $[\alpha]_D^{20} - 2370°$. The elemental analysis of the hydrochloride is consistent with the formula $C_{25}H_{33}N_3O \cdot HCl$, and the high resolution mass spectrum of the hydrochloride gives a molecular ion peak at *m/e* 391.2625 corresponding to the free base, $C_{25}H_{33}N_3O$ (*m/e* 391.2610).

The general spectroscopic properties of metacycloprodigiosin strongly suggest that it is a member of the prodigiosin series. In particular, absorptions in the visible spectrum of the free base [λ_{max}^{MeOH} (0.5% KOH) 467 nm (ϵ 31,500)] and the hydrochloride salt [λ_{max}^{MeOH} 530 nm (ϵ 75,900), λ_{sh}^{MeOH} 500 nm (ϵ 31,500)] are very similar to the corresponding absorptions of prodigiosin **3**.[5,6]

The NMR spectrum of the hydrochloride salt of metacycloprodigiosin further serves to establish the close similarity to the prodigiosins. The absorptions due to the



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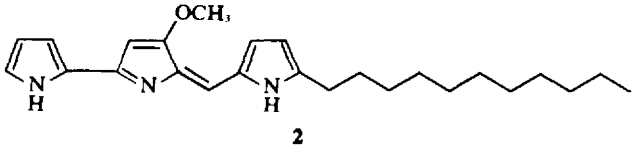

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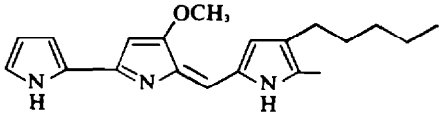

3

α,α'-bipyrrole moiety in the molecule are nearly identical with the corresponding absorptions shown by other prodigiosins.[5,6] Thus, complex multiplets at τ 2.79, 3.09 and 3.66 are assigned to the protons of ring A (see structure **1**).

Exchange of the N–H protons with D_2O causes a collapse of each multiplet to a doublet of a doublet with coupling constants typical of the 2,3,4-interaction of protons on a pyrrole ring (see Experimental).[7] The β-proton of ring B appears as a doublet (τ 3.91) which collapses to a singlet on exchange with D_2O. Furthermore, there are sharp singlets at τ 2.94 (1H) and τ 5.99 (3H) which correspond to the methylene bridge proton and the methoxyl group protons. Exchange with deuterium oxide has no effect on these absorptions. The doublet at τ 3.73 (1H), which collapses to a singlet on exchange with D_2O, is in accord with the presence of a β-proton in the dialkylpyrrole (ring C).[5,6] The alkyl portion of metacycloprodigiosin·HCl accounts for the broad absorptions at τ 6.4–7.8 (3H) and τ 7.9–9.9 (19H).

Soda-lime pyrolysis of metacycloprodigiosin

Pyrolysis of the pigment over soda-lime at reduced pressure gave a C-15 pyrrole containing 4 elements of unsaturation. Its elemental analysis and high resolution mass spectrum (molecular ion *m/e* 219.1985) are consistent with the molecular formula $C_{15}H_{25}N$ (MW 219.1980). In addition, the mass spectrum of the new pyrrole shows a loss of a methyl group at *m/e* 204 (35%) and a loss of an ethyl group at *m/e* 190 (45%). Interesting features of the mass spectrum of this compound are discussed in a later section.

The NMR spectrum clearly shows that the pyrrole is disubstituted, with one free α- and one free β-position: τ 2.67 (1H, NH), 3.70 (1H, α-ring proton), 4.19 (1H, β-ring proton). A triplet at τ 7.51 (2H, J = 6 Hz) and a multiplet at τ 7.8 (1H) are assigned to a methylene and a methine group located alpha to the pyrrole ring. A multiplet at τ 8.3–9.9 (19H) accounts for the remainder of the aliphatic protons.

The exhaustive reduction of alkylpyrroles

To complete the structure elucidation of the new pyrrole, we turned to a technique developed earlier by Thompson[8] and Beroza,[9] who showed that microgram quantities of a wide variety of organic compounds can be reduced in the gas phase at elevated temperatures to their saturated hydrocarbon backbones. Identification of the resultant hydrocarbons can aid greatly in the structure determination of the original compound. Thus, the method is ideal for structural work on natural products, many of which are available only in limited quantity. The technique has been applied to most of the major classes of compounds with hundreds of individual cases being investigated including pyrrole[9] and 2,5-dimethylpyrrole.[8]

In order to show that this method could be applied to the reduction of less volatile, branched alkylpyrroles without accompanying rearrangement, cracking or other spurious processes, we ran control experiments involving the reduction of the series of model alkylpyrroles, **4–12**.† The reductions were accomplished in a gas chromatograph fitted with a column packed with 5% platinum-on-glass. Hydrogen was employed as the carrier gas. The hydrocarbon products were collected using normal gas chromatography collection techniques (generally in yields of 65–95%) and their mass spectra compared with the mass spectra of known samples.‡ In all cases investigated, the main reduction product was identical to the hydrocarbon backbone of the original pyrrole. The results are summarized in Table 1.

The conditions of the reduction were found to be important. Thus, under milder conditions (high hydrogen flow rate, low column temperature, and high volatility of the pyrrole) incomplete reduction was observed. Under more severe conditions (low hydrogen flow rate, high column temperature, and a less volatile pyrrole) undesired side reactions were observed.§ However, conditions which yielded essentially pure hydrocarbon skeleton were found for all the pyrroles examined except **12**. In that case the expected product (70%), methylcyclododecane **13**, was accompanied by a minor amount (30%) of 3-methyldodecane **14**. Since **13** is stable to the conditions of reduction, the minor product **14** must result directly from

4: R_1 = 3-pentyl, $R_2 = R_3 = R_4$ = H
5: R_1 = cyclohexyl, $R_2 = R_3 = R_4$ = H
6: $R_1 = n\text{-}C_{11}H_{13}$, $R_2 = R_3 = R_4$ = H
7: R_2 = 3-pentyl, $R_1 = R_3 = R_4$ = H
8: R_2 = cyclohexyl, $R_1 = R_3 = R_4$ = H
9: $R_1 = R_3 = CH_3$, $R_2 = R_4$ = H
10: $R_1 = R_4 = CH_3$, $R_2 = R_3$ = H
11: $R_1 = CH_3$, $R_2 = n\text{-}C_5H_7$, $R_3 = R_4$ = H

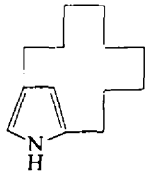

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Table 1.

Pyrrole	Column length (in.)	Column temp. (°C)	H_2 Flow rate (ml/min)	% Skeleton in product*
4	18	172	100	100
5	18	180	100	100
	9	185	60	100
6	18	188	120	95
	9	150	250	100
7	18	180	100	95
8	18	180	100	95
9	20	210	50	100
10	20	210	50	100
11	20	210	50	100
12	18	180	130	60
	9	185	230	70

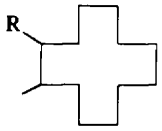
*This figure represents the percent of hydrocarbon skeleton in the product mixture.

†Pyrroles **4**, **5**, **7** and **8** were synthesized by the reaction of pyrrylmagnesium bromide with the appropriate alkyl halide (see Experimental). Pyrroles **6**[1], **11**[10] and **12**[11] are described in the literature. Pyrroles **9** and **10** were obtained from a commercial source.

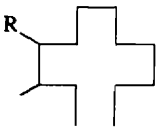
‡The alkanes required for this study were either purchased or were synthesized by standard procedures (see Experimental).

§The hydrocarbon skelton of pyrrole **4** was subjected to various conditions of reduction. Degradation was found to occur when conditions were severe enough (temperature greater than 210° and a slow hydrogen flow rate).

the reduction of pyrrole **12**. In any case, the major product resulting from the reduction of each pyrrole corresponds to the unrearranged parent hydrocarbon skeleton. Thus, it has been established that the method can be used with confidence in determining the structure of an unknown branched alkylpyrrole.



13: R = H
15: R = Et



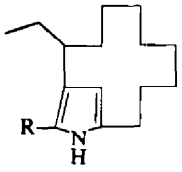
14: R = H
16: R = Et

Reduction and structure of the C-15 pyrrole

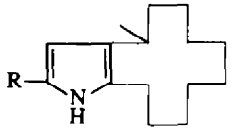
The pyrrole obtained by soda-lime pyrolysis of metacycloprodigiosin was subjected essentially to the same conditions of reduction used for **12**. A mixture of hydrocarbons consisting of 60% 1-ethyl-2-methylcyclododecane **15** and 40% 4-ethyl-3-methyldodecane **16** resulted. The mass spectra of **15** and **16** were identical with the spectra of authentic samples (see Experimental.†) It was clear that the cyclododecane **15**, the major product arising from the reduction, represented the hydrocarbon skeleton of the unknown pyrrole.

Of the $C_{15}H_{25}N$ pyrroles which may lead to **15** on reduction, only structures **17** and **18** would exhibit an NMR spectrum consistent with that observed (an α- and a β-ring proton plus protons on a methylene and methine adjacent to the pyrrole). To distinguish between these possibilities, a 2-formyl derivative was prepared for NMR examination.[12] The NMR spectrum of the formyl derivative shows the presence of a single β-ring proton, the chemical shift of which (τ4.00) is not significantly shifted from that observed for the β-proton in the parent pyrrole (τ 4.18).

Studies of model systems[5,13] show that a formyl group at the 2-position of a pyrrole ring has a pronounced deshielding effect on the adjacent hydrogen at the 3-position causing a downfield shift of about 0.8 ppm. On the other hand, the corresponding effect of a 2-formyl group on a β-hydrogen at the 4-position is very slight (0.1–0.2 ppm). The formyl derivative, therefore, clearly has structure **19**, and the C-15 dialkylpyrrole must accordingly have the novel meta-fused structure **17**.



17: R = H
19: R = CHO



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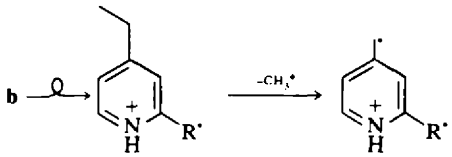
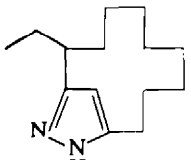
The mass spectrum of pyrrole **17**—*the* γ*-cleavage mechanism*

The mass spectrum of pyrrole **17** exhibits peaks at *m/e* 204 (35% relative intensity, loss of methyl) and *m/e* 190 (40% relative intensity, loss of ethyl). On first consideration, the M^+-15 peak, ostensibly resulting from fission of a bond γ-to the pyrrole ring, seems exceptional. It is, however, readily explained in terms of the sequence shown in Scheme 1. Cleavage of one of the bonds β- to the pyrrole ring leads to loss of an ethyl group along with the ion **d**. Cleavage of the alternative β-bond leads to the ion **b**, from which loss of a methyl group yields the stabilized ion **c**.‡

†We thank Dr. J. Nadelson for the preparation of compound **15**.

‡Alternatively, ion **b** may first rearrange to the pyridinium ion **e**, which could also undergo loss of a methyl group leading to the relatively stable ion **f**.





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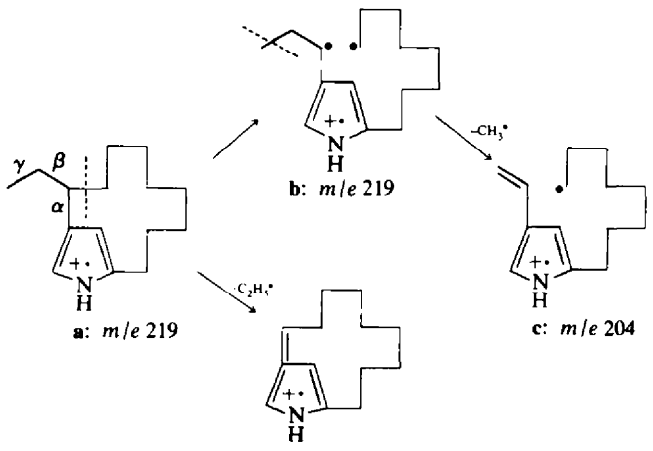
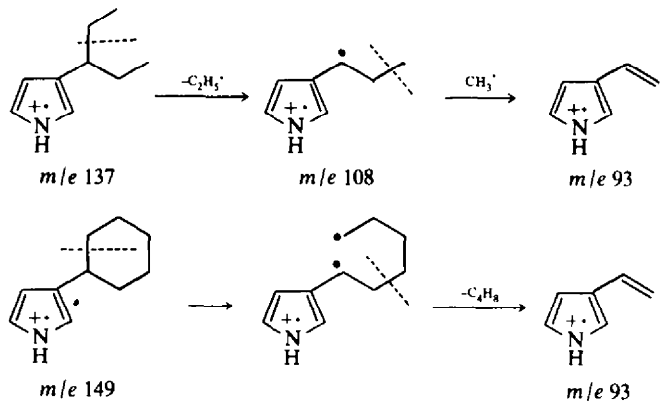
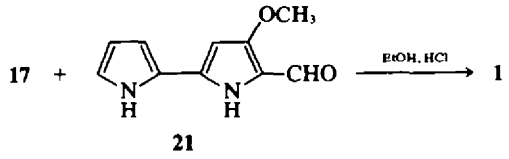


Scheme 1.



Scheme 2.



As would be predicted by the above mechanism, the mass spectrum of 3 - (3 - pentyl)pyrrole **7** exhibits peaks at *m/e* 137, 108 and 93, and the mass spectrum of 3-cyclohexylpyrrole **8** exhibits peaks at *m/e* 149 and 93 (see Scheme 2). In addition, the pyrazolophane **20**[4] shows loss of methyl and ethyl fragments in direct analogy to the meta-fused pyrrole **17**. The γ-cleavage mechanism as illustrated in Schemes 1 and 2 thus seems to represent a general cleavage pattern.

Reconstitution of metacycloprodigiosin

As a member of the prodigiosin series, metacycloprodigiosin **1** would be expected to be formed by condensation of precursors corresponding to pyrrole **17** and the methoxybipyrrole aldehyde **21**.[14-16] In accord with expectation, the parent pigment was reconstituted by the HCl-catalyzed condensation of the alkylpyrrole **17** with the C-10 prodigiosin precursor **21**, obtained from a mutant strain of *Serratia*.[17] This reaction yielded metacycloprodigiosin hydrochloride identical (NMR, IR, MS) with the natural material.

EXPERIMENTAL

M.ps and b.ps are uncorrected. IR spectra were recorded on either a Perkin–Elmer, Model 421 Recording Infrared Spectrometer or a Perkin–Elmer, Model 237 Grating Spectrophotometer. Carbon tetrachloride was used as the solvent unless otherwise noted. NMR spectra were taken on a Varian Model A60-A Spectrometer. Chemical shifts are reported in τ units using TMS as internal standard. Mass spectra were recorded either on a CEC, Model 21–103 Mass Spectrometer, or on a AEI, Model MS-9 instrument. UV spectra were recorded on a Cary, Model 11-S or on a Bausch and Lomb Model 550 recording spectrophotometer. Optical rotations were recorded on a Stanley Photoelectric Polarimeter. An average of 4 readings is reported in each instance. GLC analyses and sample collections were obtained with a Varian Aerograph, Model A90-P3 instrument. A 5′ × 1/4″ 20% Silicon Gum Rubber (SE-30) on 60/80 mesh Chromosorb W column was used for analytical purposes, and a 8′ × 3/8″ 20% Silicon Gum Rubber (SE-30) on 60/80 mesh Chromosorb W column was used for preparation purposes unless otherwise noted.

Isolation of pigment

Streptomyces longisporus ruber strain M-3 was grown on a soymeal-mannitol medium as previously described.[1] The mixture of pigments **1** and **2** was separated after extraction, washing and chromatography.

Isolation of metacycloprodigiosin **1**

The crude, dark-red hydrochloride (1 g) was dissolved in a small amount of methylene chloride and washed with 1 N NaOH. The solution was dried (Na_2SO_4), the solvent removed *in vacuo*, and the pigment redissolved in a small volume of petroleum ether. This solution was applied to a column of basic alumina (40 g of Fisher, Brockman Activity 1) packed in petroleum ether. The column was developed with 250 ml portions of solvent, starting with petroleum ether and changing in 10% increments through the series: petroleum ether, petroleum ether/chloroform. A pink (minor component) band was eluted with 10% chloroform.

The main pigment band was eluted with 30% chloroform, resulting in a partial separation of the two pigments. By removing the band in 50 ml fractions, it was possible to obtain the faster moving component, metacycloprodigiosin **1**, free from the second component **2**. The new pigment was obtained as orange-brown crystals from petroleum ether: m.p. 208–209°; $[\alpha]_D^{20} - 2370°$; λ_{max} (CH_3OH + 0.5% KOH) 467 nm (ε 30,600), 325 (4,850), 285 (6,050); IR ($CHCl_3$) 3010, 2975, 2950, 2875, 1620, 1602, 1548, 1452, 1368, 1280, 1125, 1063, 968 cm^{-1}; NMR ($CDCl_3$) τ −0.6 (broad, 2H), 3.05 (s, 1H), 3.36 (m, 2H), 3.87 (m, 1H), 3.94 (s, 1H), 4.08 (s, 1H), 6.04 (s, 3H), 7.13 – 8.06 (m, 3H), 3.13 – 9.42 (m, 19H).

The remainder of the band contained a mixture of pigments **1** and **2**. It was washed with 1 N HCl, dried (Na_2SO_4), and concentrated *in vacuo* yielding a dark-red, amorphous solid. Crystallization of the solid from CCl_4 yielded pure **1**·HCl. The isolation and crystallization of **2** is described in the previous paper.[1]

Metacycloprodigiosin hydrochloride **1**·HCl

A solution containing 250 mg (0.64 mmol) of **1** in 100 ml of CH_2Cl_2 was washed with 100 ml of 1N HCl. The organic layer was separated and dried (Na_2SO_4). The solvent was removed *in vacuo* and the residue crystallized from carbon tetrachloride to yield 250 mg (90%) of metacycloprodigiosin hydrochloride as long, plate-like crystals which were red to transmitted light and green to reflected light: m.p. 218–220°; λ_{max} (CH_3OH) 530 nm (ε 75,900), 500 (sh) (31,500), 362 (5,000), 297 (6,900), 273 (3,640); IR ($CHCl_3$) 3185, 2995, 2970, 2940, 2863, 1629, 1601, 1570, 1546, 1520, 1419, 1382, 1279, 1268, 1239, 1187, 1166, 1135, 1068, 1045, 1010, 961 cm^{-1}; NMR ($CDCl_3$) τ-2.7 (broad, 3H), 2.79 (m, 1H), 2.94 (s, 1H), 3.09 (m, 1H), 3.66 (m, 1H), 3.73 (d, 1H), 3.91 (d, 1H), 5.99 (s, 3H), 6.4–7.8 (m, 3H), 7.9–9.9 (m, 19H); MS *m/e* (rel intensity) 393 (4), 392 (30), 391 (100), 376 (10), 320 (10), 307 (16), 175 (12). MW; Calc. for $C_{25}H_{33}N_3O$ (free base): 391.2610. Found (high-resolution MS): 391.2625. (Found: C, 69.89; H, 8.10; N, 9.53; Cl, 8.08. Calc. for $C_{25}H_{33}N_3O$. HCl: C, 70.12; H, 8.02; N, 9.81; Cl, 8.28).

N,N′-Dideuterometacycloprodigiosin deuterochloride

A sample of metacycloprodigiosin hydrochloride (40 mg) was

dissolved in deuterochloroform (350 μl). The solution was transferred to an NMR tube and approximately 1 ml of D_2O was added along with a trace of hydrogen chloride gas. After the tube was shaken vigorously, the NMR spectrum of the sample was recorded. It was observed that not all of the N-protons had exchanged. A minute portion of potassium acetate was then added, and the tube shaken vigorously. The NMR spectrum now showed that all 3 N-protons had been exchanged for deuterium: τ 2.78 (dd, 1H, J = 1.2 Hz, 2.3 Hz), 2.95 (s, 1H), 3.08 (dd, 1H, J = 1.3 Hz, 3.8 Hz), 3.66 (dd, 1H, J = 2.3 Hz, 3.8 Hz), 3.72 (s, 1H), 3.91 (s, 1H), 5.98 (s, 3H), 6.5–7.8 (m, 3H), 8.0–9.9 (m, 19H).

Pyrolysis of metacycloprodigiosin **1**

A sample of metacycloprodigiosin hydrochloride (219 mg; 0.51 mmol) was intimately ground with soda-lime (3.95 g) which had been dried for 2 h over a Bunsen burner. This mixture was then placed in a pyrolysis tube (open at both ends) over a glass wool plug and 1" of pure soda-lime. The tube was sealed at the end nearest to the pigment—soda lime mixture, evacuated to 0.1 mm or less and maintained at reduced pressure for 1 h. The tube, still under vacuum, was then slowly pushed into a tube heater at 450° so that the pyrolysate would have to distill first through the soda-lime and then the glass wool plug. When half of the soda-lime plug was inserted into the tube heater, the pyrolysis tube was allowed to stand for 10 min (when the pyrolysis tube was not allowed to stand several side products were observed). The entire tube was then inserted into the heater. In a few minutes, 48 mg (44%) of a yellow oil which partially solidified on standing, condensed on the walls of the pyrolysis tube. Preparative GLC followed by sublimation gave a pure sample of **17**: m.p. 48–54°; IR (CCl_4) 3460 (sharp), 3360 (broad), 2925, 2850, 1485, 1460, 680 cm^{-1}; NMR (CCl_4) τ 2.7 (broad, 1H), 3.70 (t, 1H), 4.19 (t, 1H), 7.51 (t, 2H), 7.8 (m, 1H), 8.27–9.9 (m, 19H); MS *m/e* (rel intensity) 220 (18), 219 (100), 218 (9), 204 (35), 190 (40), 176 (19), 162 (15), 148 (14), 134 (25), 120 (25), 106 (33), 80 (26). MW; Calc. for $C_{15}H_{25}N$: 219.1980. Found (high-resolution MS): 219.1985.

The exhaustive reduction of model pyrroles

A. The pyrroles. The pyrroles were either purchased (**9** and **10**), synthesized by a route already recorded in the literature (**6**[1], **11**[10,16] and **12**[11]), or were synthesized by the procedure developed by Skell and Bean[18] for the preparation of alkyl pyrroles (**4**, **5**, **7** and **8**). The properties of the latter pyrroles are listed below.

2 - (3 - Pentyl)pyrrole **4** *and 3 - (3 - pentyl)pyrrole* **7**. A mixture of these pyrroles was prepared by the reaction of 3-bromopentane[19] with pyrrylmagnesium bromide.[18] The mixture was subjected to preparative GLC (12' × 3/8" column packed with 15% carbowax 20 M on 70/80 mesh Anakrom ABS; column temp. 200°). The compound with the shorter retention time was **4**: IR (film) 3480, 3375, 3090, 2960, 2930, 2870, 1465, 1020, 780, 710 cm^{-1}; NMR (CCl_4) τ 2.5 (broad, 1H), 3.54 (m, 1H), 4.02 (q, 1H), 4.22 (m, 1H), 7.74 (m, 1H), 8.56 (m, 4H), 9.19 (τ, 6H); MS *m/e* (rel intensity) 137 (22), 108 (100), 93 (16), 80 (23). (Found: C, 78.74, H, 11.10; N, 10.33. Calc. for $C_9H_{15}N$: C, 78.83; H, 10.95; N, 10.22%).

The compound with the longer retention time was **7**: IR (film) 3485, 3400, 2955, 2920, 2870, 1455, 1060, 750 cm^{-1}; NMR (CCl_4) τ 2.5 (broad, 1H), 3.52 (q, 1H), 3.68 (q, 1H), 4.06 (q, 1H), 7.80 (m, 1H), 8.56 (m, 4H), 9.19 (τ, 6H); MS *m/e* (rel intensity) 137 (25), 108 (100), 93 (14), 80 (34). (Found: C, 78.80; H, 10.82; N, 10.25. Calc. for $C_9H_{15}N$: C, 78.83; H, 10.95; N, 10.22%).

2-Cyclohexylpyrrole **5** *and 3-cyclohexylpyrrole* **8**. A mixture of pyrroles **5** and **8** was prepared by the reaction of cyclohexyl bromide with pyrrylmagnesium bromide.[18] The mixture was subjected to preparative GLC (12' × 3/8" column packed with 15% Carbowax 20M on 70/80 mesh Anakrom ABS; column temp. 200°). The compound with the shorter retention time was **5**: IR (CCl_4) 3500, 3400, 3100, 2920, 2850, 1450, 1095, 1025 cm^{-1}; NMR (CCl_4) τ 2.60 (broad, 1H), 3.58 (q, 1H), 4.05 (q, 1H), 4.24 (m, 1H), 7.7 (m, 1H), 8.0–9.1 (m, 10H); MS *m/e* (rel intensity) 149 (49), 120 (9), 106 (100), 93 (21), 80 (38). (Found: C, 80.62; H, 10.19; N, 9.48. Calc. for $C_{10}H_{15}N$: C, 80.54; H, 10.07; N, 9.40%).

The compound with the longer retention time was **8**: IR (CCl_4) 3490, 3400, 3100, 2920, 2850, 1450, 1060, 951 cm^{-1}; NMR (CCl_4) τ 2.60 (broad, 1H), 3.55 (m, 1H), 3.70 (m, 1H), 4.04 (q, 1H), 7.6 (m, 1H), 8.0–9.1 (m, 10H); MS *m/e* (rel intensity) 149 (58), 120 (14), 106 (100), 93 (29), 80 (45). (Found: C, 80.48; H, 10.24; N, 9.47. Calc. for $C_{10}H_{15}N$: C, 80.54; H, 10.07; N, 9.40%).

B. Hydrocarbon skeletons

The hydrocarbons corresponding to the carbon skeletons of pyrroles **4–12** were either purchased (carbon skeletons corresponding to pyrroles **4–6**, **8–11**) or were synthesized by the reaction of a Grignard or alkyllithium reagent with an appropriate ketone, followed by dehydration and hydrogenation (carbon skeletons corresponding to pyrroles **7** and **12**). In addition, 3-methyldodecane, a minor side-product arising from the reduction of pyrrole **12**, was synthesized by such a route. The following experimental details provided for one of these syntheses will serve to illustrate the methods used for making these hydrocarbons. The properties of the other hydrocarbons are also given.

3 - Ethyl - 4 - methylhexane (the carbon skeleton of **7**). A solution consisting of 3.1 g (0.055 mol) of *sec*-butyllithium (Alfa Inorganics, Inc.) in pentane was placed in a 500 ml, 3-neck, round-bottomed flask equipped with a nitrogen inlet tube, mechanical stirrer, addition funnel, and a reflux condenser. A solution of 3-pentanone (4.8 g, 0.056 mol) in pentane (100 ml) was added at a rate sufficient to maintain a gentle reflux. After addition was complete the reaction mixture was heated at reflux for 0.5 h. the solution was then cooled and poured into 200 ml of saturated ammonium chloride solution. The layers were separated and the aqueous layer extracted with two portions of ether (100 ml). The organic layers were combined, washed with two portions of water (100 ml), and then one portion of NaCl soln (100 ml). After the solution was dried ($MgSO_4$), the solvent was removed by distillation (12" Vigreux column) to yield crude 3 - ethyl - 4 - methyl - 3 - hexanol (7.4 g, 90%). A portion of the crude alcohol was purified by preparative GLC (165°) IR (film) 3600, 3460, 2950, 2925, 2875, 1470, 1390, 950 cm^{-1}; NMR (CCl_4)τ 8.6 (m, 7H), 9.1 (m, 13).

Phosphorus oxychloride (12.5 g, 0.083 mol) was added cautiously to a solution of 3 - ethyl - 4 - methyl - 3 - hexanol in pyridine (38 g, 0.48 mol) cooled to 0°. The mixture was heated at 100° for 3.5 h, cooled, and poured into 100 ml of water. Conc HCl (45 ml) was added, to form pyridine hydrochloride, and the mixture was extracted with ether for 30 h in a liquid–liquid continuous extractor. The extract was washed with 100 ml of 1N sodium bicarbonate solution, 100 ml of water, and 100 ml of sat NaCl soln. After the solution was dried ($MgSO_4$), the solvent was removed by distillation (12" Vigreux column) to yield a mixture of 3 - ethyl - 4 - methylhexenes (5.3 g, 82%). A portion of the crude mixture of olefins was purified by preparative GLC (12" × 3/8" column packed with 15% Carbowax 20M on 70/80 mesh Anakrom ABS; column temp. 120°). IR (film) 2970, 2940, 2870, 1460, 1370, 825 cm^{-1}; NMR (CCl_4) τ 4.9 (m), 7.5 (m), 8.1 (m), 8.4 (d), 8.7 (m), 9.1 (m). (Found: C, 85.71; H, 14.17. Calc. for C_9H_{18}: C, 85.63; H, 14.3%).

The mixture of 3-ethyl-4-methylhexene isomers (5 g, 0.04 mol) was dissolved in ethyl acetate/acetic acid (50:50, 10 ml) and three drops of conc. HCl. This solution was stirred with 5% platinum-on-charcoal (150 mg) under hydrogen for 96 h. It was then filtered, poured into a stoichiometric amount of 1N NaOH, and extracted with three 100 ml portions of pentane. The combined extracts were washed with 100 ml of water and dried ($MgSO_4$). Removal of the pentane by distillation (12" Vigreux column) left a residue of crude 3 - ethyl - 4 - methylhexane (3 g, 60%). The product was further purified by preparative GLC (12' × 3/8" column packed with 15% Carbowax 20 M on 70/80 mesh Anakrom ABS; column temp. 110°): IR (film) 2960, 2925, 2870, 1470, 1385 cm^{-1}; NMR (CCl_4) τ 8.8 (m), 9.1 (m); MS *m/e* (rel intensity) 128 (3), 99 (18), 70 (70), 57 (100). (Found: C, 84.33; H, 15.49. Calc. for C_9H_{10}: C, 84.28; H, 15.72%).

Methylcyclododecane (the carbon skeleton of **12**). The hydrocarbon was synthesized by the reaction of methylmagnesium iodide with cyclododecanone, followed by dehydration of the resultant alcohol with phosphorous oxychloride, and catalytic reduction of the olefin mixture. The experimental conditions were similar to those described above for the synthesis of 3 - ethyl - 4 - methylhexane. The crude product was purified by preparative GLC (180°): IR (film) 2940, 2860, 1470, 1440, 1370 cm^{-1}; NMR

(CCl_4) τ 8.7 (s, 23H), 9.1 (d, 3H); MS *m/e* (rel intensity) 182 (35), 167, (2), 154 (12), 125 (18), 111 (31), 97 (52), 83 (73), 69 (79), 55 (100). Found: C, 85.80; H, 14.18. Calc. for $C_{13}H_{26}$: C, 85.63; H, 14.37%).

3-Methyldodecane (minor product from the reduction of **12**). 3-Methyldodecane was synthesized by reaction of methylmagnesium iodide with 3-dodecanone (Chemical Samples Co.) followed by dehydration of the resultant alcohol with phosphorous oxychloride, and catalytic reduction of the olefin mixture. The experimental conditions were similar to those described for the synthesis of 3 - ethyl - 4 - methylhexane. The crude product was purified by preparative GLC: IR (film) 2950, 2920, 2850, 1460, 1370 cm^{-1}; NMR (CCl_4) τ 8.7 (s, 19H), 9.1 (t, 9H); MS *m/e* (rel intensity) 184 (1.5), 169 (1), 155 (16), 154 (11), 126 (4), 113 (7), 99 (16), 85 (35), 71 (45), 57 (100). (Found: C, 85.03; H, 14.88. Calc. for $C_{13}H_{28}$: C, 84.69; H, 15.21%).

C. Preparation of 5% platinum-on-glass reduction columns

To a solution consisting of 0.66 g of chloroplatinic acid (0.25 g of Pt) in 10 ml water was added 5 g of 30 mesh ground vycor glass. The water was removed *in vacuo*. The remaining powder was dried at 100° for 24 h and then packed into 1/4 in aluminium tubing. The column was mounted in an Aerograph model A-90 GLC instrument (single effluent port). The column temperature was maintained at 213° while hydrogen was passed through the column at a slow rate. This process was continued until no more hydrogen chloride gas was eluted from the column (2 days). Several reduction columns of varying lengths were prepared following this procedure.

D. Reduction of the model alkylpyrroles

A solution of the alkylpyrrole in an equal volume of acetone was added at a rate of 3–5 μl/min to the reduction column, and the reduction products were collected by standard trapping techniques used in preparative gas chromatography. The products were analyzed by GLC and, if necessary, were separated and purified by preparative GLC. The products were identified by comparison of their mass spectra with the mass spectra of authentic samples. The data for the reduction of each pyrrole are presented in summary form in Table 1.

Reduction of the meta-fused pyrrole **17**. A solution of 45 mg of **17** in 50 ml of acetone was injected onto an 18" 5% platinum-on-glass column (mounted in the gas chromatograph described above) maintained at 180°. The hydrogen flow rate was 130 ml/min and the products of reduction were collected in a trap immersed in an ice-bath. The products were separated by preparative GLC. The major product (60%) was identical (mass spectrum) to 1 - ethyl - 2 - methylcyclododecane **15**; the minor product (40%) was identical (mass spectrum) to 4-ethyl-3-methyldodecane **16**. The synthesis of these hydrocarbons is described in a subsequent section.

Treatment of an acetone solution of 4-ethyl-3-methyldodecane **15** under the same conditions used to reduce **17** resulted in complete recovery of the hydrocarbon. Under more severe conditions (18" column, column temp. 215°, hydrogen flow rate 70 ml/min), some decomposition occurred (60–80% of **15** recovered).

1-Ethyl-2-methylcyclododecane **15**. A solution of 2-methylcyclododecanone[20] (5 g, 0.025 mol) in ether was added to a solution of ethylmagnesium bromide (prepared from 5.5 g of ethyl bromide and 1.2 g of magnesium) at a rate sufficient to maintain gentle reflux. The reaction mixture was heated at reflux for 1 h, cooled, and poured into 100 ml of saturated ammonium chloride solution. The organic layer was washed with two portions of water (100 ml), and one portion of NaCl soln (100 ml). After the solution was dried ($MgSO_4$), the solvent was removed *in vacuo*, and the crude product distilled (b.p. 105–110°/2.5–3.0 mm) to yield 4.39 g (84%) of 1 - ethyl - 2 - methylcyclododecanol: IR (film) 3610, 3475, 2940, 2870, 1475, 1460, 1380, 1360 cm^{-1}; NMR (CCl_4) τ 8.6 (m, 23 H), 9.1 (m, 7 H). (Found: C, 79.39; H, 13.41. Calc. for $C_{15}H_{30}O$; C, 79.64; H, 13.27%).

Phosphorus oxychloride (3.0 g, 0.02 mol) was added cautiously to a solution of 1-ethyl-2-methylcyclododecanol (3.1 g, 0.0138 mol) in pyridine. The mixture was heated at 100° for 5 h, cooled, and poured into 10% HCl. The aqueous mixture was extracted with ether, and the ether extracts washed with $NaHCO_3$ soln. After drying ($MgSO_4$), the solvent was removed *in vacuo* to yield a crude mixture of olefins (2.1 g, 50%). A sample of the $C_{15}H_{28}$ olefins was purified by preparative GLC. (Found: C, 86.30; H, 13.62. Calc. for $C_{15}H_{28}$: C, 86.52; H, 13.48%).

The mixture of 1-ethyl-2-methylcyclododecane isomers (2.1 g 0.01 mol) was dissolved in ethanol-acetic acid (1:1, 50 mol). This solution was hydrogenated on a Paar shaker (55 psi, 25°, 96 h) using a platinum oxide catalyst (200 mg). The reaction mixture was filtered, poured into a stoichiometric amount of 1N NaOH, and extracted with three 100 ml portions of ether. The combined extracts were washed with 100 ml of water and dried ($MgSO_4$). Removal of the ether *in vacuo* left a residue which was further purified by preparative GLC to yield 1-ethyl-2-methylcyclododecane **15**: NMR (CCl_4) τ 8.65 (m, 26H), 9.15 (m, 6H); MS *m/e* (rel intensity) 211 (6), 210 (26), 182 (4), 181 (3), 126 (5), 125 (8), 112 (8), 111 (19), 98 (12), 97 (39), 84 (25), 83 (44), 70 (39), 69 (55), 57 (42), 56 (54), 55 (100). (Found: C, 85.60%; H, 14.23. Calc. for $C_{15}H_{30}$: C, 85.71; H, 14.30%).

4 - Ethyl - 3 - methyldodecane **16**. A solution consisting of 15.5 g (0.091 mol) of 3-undecanone (Chemical Samples Co.) in pentane (100 ml) was added to a solution containing 5.8 g (0.091 mol) of *sec*-butyllithium (Alfa Inorganics, Inc.) in hexane (70 ml) at a rate sufficient to maintain a gentle reflux. The reaction mixture was heated at reflux for 1 h, cooled, and the product isolated in a manner similar to that described earlier for the preparation of 1 - ethyl - 2 - methylcyclododecanol. A portion of the crude 4 - ethyl - 3 - methyl - 4 - dodecanol (17.5 g, 86%) was purified by preparative GLC: IR (film) 3625, 3480, 2950, 2850, 1460, 1375 cm^{-1}; NMR (CCl_4) τ 8.7 (s, 19H), 9.1 (m, 13H).

Phosphorus oxychloride (17.5 g, 0.115 mol) was cautiously added to a solution of 4 - ethyl - 3 - methyl - 4 - dodecanol (17 g, 0.077 mol) in pyridine (70 ml). The mixture was heated at 100° for 4 h, cooled, and subjected to a work-up similar to that described previously for the preparation of 1 - ethyl - 2 - methylcyclododecane. A sample of the crude mixture of olefins (11.5 g, 70%) was purified by preparative GLC: IR (film) 2960, 2930, 2850, 1460, 1380 cm^{-1}; NMR (CCl_4) τ 4.9 (m), 7.5 (m), 8.1 (m), 8.4 (d), 8.7 (broad, s), 9.1 (m). (Found: C, 85.72; H, 14.15. Calc. for $C_{15}H_{30}$: C, 85.63; H, 14.37%).

The mixture of olefins (7.8 g, 0.037 mol) was dissolved in ethyl acetate–acetic acid (75:25, 100 ml) and 2 drops of conc HCl. The solution was hydrogenated on a Paar shaker (46 psi, 55°, 8 h) using platinum oxide (250 mg) as the catalyst. The product was isolated (7.0 g, 90%) in a manner similar to that described for 1 - ethyl - 2 - methylcyclododecane. A portion of the crude 4 - ethyl - 3 - methyldodecane was purified by preparative GLC (12' × 3/8" column packed with 15% Carbowax 20 M on 70/80 mesh Anakrom ABS; column temp. 150°): IR (CCl_4) 2960, 2920, 2850, 1460, 1380 cm^{-1}; NMR (CCl_4) τ 8.7 (broad s, 20 H), 9.1 (m, 12 H); MS *m/e* (rel intensity) 212 (0.5), 183 (5), 155 (12), 154 (23), 99 (15), 85 (23), 71 (44), 57 (100). (Found: C, 85.13; H, 15.01. Calc. for $C_{15}H_{32}$: C, 84.82; H, 15.18%).

Formylation of the meta*-fused pyrrole* **17**. The method of Silverstein *et al.* was followed.[12] Dimethylformamide (33 mg, 0.453 mmol) was treated with 70 mg (0.453 mmol) of phosphorous oxychloride at 0° in a flask equipped with a magnetic stirrer. The reaction was then allowed to warm to room temp. and remain for 20 min. The meta-fused pyrrole **17** (90 mg, 0.411 mmol) obtained from pyrolysis and GLC purification was then added in 2 ml of ethylene dichloride. This addition was carried out dropwise with stirring. The reaction was heated at reflux temp. for 15 min and then allowed to cool to room temperature. A solution of 40 mg of sodium acetate trihydrate in 0.6 ml of water was then added and the solution was heated at reflux temp. an additional 20 min. After cooling and slight dilution with water and ethylene dichloride, the layers were separated. The water layer was wahsed three times with ethylene dichloride and the combined organic layers were then washed three times with sat Na_2CO_3 soln. The organic layer was then dried (K_2CO_3) and the solvent removed, giving 90 mg of a brown solid. The NMR spectrum of the reaction product showed it to be entirely free of starting material or other major impurities. The crude material was chromatographed on undeactivated silica

gel using a pentane/ether solvent system, elution being accomplished with 30% ether. On removal of the solvent, yellow needles resulted. These were recrystallized from ethanol/water yielding the formyl pyrrole **19**: m.p. 109–112°; NMR (CCl_4) τ −1.50 (broad, 1 H), 0.65 (s, 1 H), 4.0 (s, 1 H), 7.3 (m, 3 H), 8.0–915 (m, 19 H). (Found: C, 77.49; H, 9.97; N, 5.64. Calc. for $C_{16}H_{25}NO$: C, 77.68; H, 10.19; N, 5.66%).

Reconstitution of metacycloprodigiosin 1. A solution containing 95 mg (0.43 mmol) of meta-fused pyrrole **17**, 76 mg (0.4 mmol) of the bipyrrole precursor **21**[14 16] in 100 ml of ethanol was treated with 1 drop of conc. HCl and allowed to stand for 42 h. The solvent was then removed, the pigment converted to its basic form, and chromatographed on basic alumina using a petroleum ether/chloroform solvent system. The effluent was washed with 1N HCl and the solvent removed, yielding 54 mg of a dark solid. The hydrochloride was then resubjected to the same process of purification, again giving a dark solid, m.p. 201.5–202.5°. The spectroscopic properties (IR, UV and NMR) of the reconstituted pigment were identical to those of the natural pigment. (Found: C, 70.07; H, 8.00; N, 9.64; Cl, 8.25. Calc. for $C_{25}H_3N_3O$·HCl: C, 70.15; H, 8.01; N, 9.82; Cl, 8.28%).

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