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UNDECYLPRODIGIOSIN

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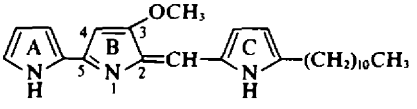
Abstract—Full details are provided on the proof of structure of undecylprodigionsin, a tripyrrole pigment isolated from *Streptomyces longisporus ruber.*

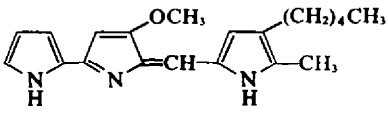
Among the naturally occurring prodigiosins[1] which have been isolated in recent years are the C-25 pigments from various actinomycetes.[2] One of these prodigiosin analogs which we have named undecylprodigiosin† has been described in our earlier preliminary communication[3] and in a report by Harashima.[4] Based on spectroscopic, degradative, and synthetic studies we assigned structure 1 to this pigment. In this paper we have provided full details of our investigation on the isolation, structure determination and synthesis of this compound.



1

Streptomyces longisporus rubber, 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 consisted of metacycloprodigiosin, a prodigiosin-like pigment, the structure and synthesis of which is described in accompanying papers.[5] The second fraction contained a mixture of metacycloprodigiosin and undecylprodigiosin 1. Treatment of the mixture with hydrochloric acid, followed by fractional crystallization from carbon tetrachloride and then repeated crystallizations from heptane yielded pure 1 as the hydrochloride salt. The crystalline hydrochloride exhibits dimorphism, melting at 76–78° and 106–107°. A crystalline zinc derivative, m.p. 102–103°, gave elemental analysis consistent with the formula $(C_{25}H_{34}N_3O)_2Zn$. The high resolution mass spectrum of the hydrochloride of 1 shows a molecular ion peak at *m/e* 393.2781 corresponding to the free base, $C_{25}H_{35}N_3O$ (*m/e* 393.2768).

The general spectroscopic properties of undecylprodigiosin 1 clearly show it to be a member of the prodigiosin series. In particular, absorptions in the visible spectrum of the free base [$\lambda_{Max}^{MeOH+0.5\%\ KOH}$ 462 mμ (ε 40,200), $\lambda_{Sh}^{MeOH+0.5\%\ KOH}$ 530 mμ (7000)] and hydrochloride salt [λ_{Max}^{MeOH} 530 mμ (ε 101,000), λ_{Sh}^{MeOH} 500 mμ (ε 50,000)] are almost identical in wave length and intensity to the corresponding absorptions of prodigiosin 2.[6,7]



2

The mass spectrum of undecylprodigiosin contains a parent peak at *m/e* 393 and a strong peak at *m/e* 252 (loss of $C_{10}H_{21}$). There is also a significant peak at *m/e* 238 (24%) corresponding to loss of $C_{11}H_{23}$. According to Jackson *et al.* one of the most intense ions in the spectrum of prodigiosin 2 results from β-cleavage of the alkyl side chain. Jackson also points out that alkylpyrrylmethenes can suffer α-cleavage of their side chains, although the latter process is considerably less favorable than the former.[8,9] The observed substantial loss of $C_{10}H_{21}$ accompanied by a significant loss of $C_{11}H_{23}$ in the mass spectrum of undecylprodigiosin is thus indicative of an undecyl side chain. These conclusions are supported by the NMR spectrum of the hydrochloride salt of 1 showing absorptions at τ 9.13 (t, 3 H), 8.76 (m, 18 H) and 7.06 (t, 2 H) in accord with the presence of an *n*-undecyl side chain.

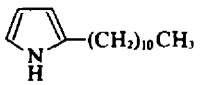
The NMR spectrum also contains a sharp singlet methoxyl group absoprtion at τ 6.05 and a broad NH absorption τ −2.83 (3 H). The absorption due to the latter protons disappears on exchange with deuterium oxide. A sharp singlet at τ 3.06 (1 H) is assigned to the proton at the methylene bridge position between rings B and C (see structure 1). Exchange with deuterium oxide has no effect on this absorption. A doublet at τ 3.96 (1 H) which collapses to a singlet in the N-deuterated pigment may best be associated with the proton adjacent to the methoxyl group in ring B at C-4. Three complex multiplets at τ 2.78, 3.10 and 3.67 integrating for one proton each are assigned to three protons on the monosubstituted pyrrole (ring A). Complex multiplets at τ 3.17 and 3.80 integrating for one proton each are assigned to the pyrrole(ring C) bearing the undecyl substituent. In the N-deuterated pigment these multiplets become an AB-quartet. The coupling constant, J = 3.8 Hz, is that expected for 3.4-interaction in the pyrrole ring.[10]

The above evidence clearly indicates that there is an undecyl group located at an α-position of one of the pyrrole rings. By analogy with prodigiosin 2 one would expect this alkyl substitution to be located on ring C. Conclusive proof for the location of the undecyl side chain was obtained by treatment of the pigment with strong base at elevated temperature, as described below, a reaction which is known to eliminate the monopyrrole fragment from the parent pyrryldipyrrylmethene.[6,12]

Soda lime pyrolysis at reduced pressure of undecylprodigiosin hydrochloride gave a basic substance whose elemental analysis is consistent with the formula $C_{15}H_{27}N$.

†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).

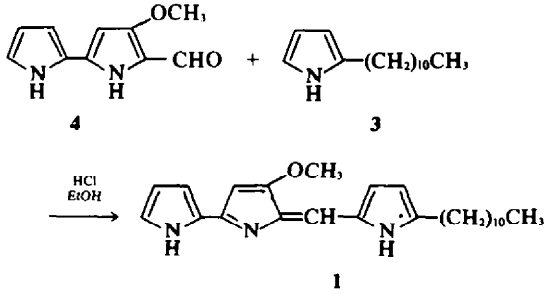
‡The strains of *Streptomyces longisporus ruber*, strain M-3, were kindly provided by Dr. K. Haider, Institut fur Biochemie des Bodens, Braunschweig, Germany.



3

The mass spectrum of their base exhibits a parent ion at *m/e* 221 (46%) and a loss of $C_{10}H_{21}$ at *m/e* 80 (100%). Its NMR spectrum has a broad absorption at τ 2.55 (1 H, NH), a multiplet at τ 3.58 (1 H, α-ring proton), a quartet at τ 4.07 (1 H, β-ring proton), a multiplet at τ 4.24 (1 H β-ring proton), a triplet at τ 7.5 (2 H, aromatic methylene), a broad singlet at τ 8.75 (18 H, aliphatic methylenes) and a triplet at τ 9.11 (3 H, aliphatic methyl). The above data are in accord with the 2-*n*-undecylpyrrole structure **3**. This assignment was confirmed by comparison of the base (IR, NMR and MS) with an authentic sample of the compound prepared by the reaction of pyrrylmagnesium bromide with *n*-undecyl bromide.[11]

By analogy with prodigiosin **2** which yields 3-amyl-2-methylpyrrole on soda-lime pyrolysis, undecylprodigiosin should be represented by structure **1**, and this was established by partial synthesis. Condensation of 2-*n*-undecylpyrrole **3** with the methoxy bipyrrole aldehyde **4**[6,13-15] in ethanolic hydrochloric acid yielded a synthetic product identical in all respects with the natural pigment. Since the bipyrrole aldehyde **4** is of known constitution,[13,15] and has previously been synthesized,[15] the structure of undecylprodigiosin is confirmed as **1**.



Harashima *et al.* have isolated a C-25 homologue of prodigiosin **2** which they designated as prodigiosin 25-C.[4] Direct comparison of undecylprodigiosin **1** with prodigiosin 25-C (m.m.p., IR, TLC has shown that they are identical.†

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. 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 on an AE1, Model MS-9 instrument. Gas-liquid chromatographic (GLC) analyses and sample collections were effected with a Varian Aerograph, Model A90-P3 instrument. A 5′ × ¼″ 20% Silicon Gum Rubber (SE-30) on 60/80 mesh Chromosorb W column was used for preparative purposes, unless otherwise noted.

Growth of Streptomyces longisporus ruber, *strain M-3*

The cultures were grown in 2.8-l Fernbach flasks containing 500 ml of medium. The medium was prepared by boiling a mixture of 2% mannitol and 3.5% soymeal in tap water for 10 min, letting the suspension settle for 20–30 min and then removing the supernatent solution by decantation. The resultant cloudy solution was autoclaved at 120° for 20–30 min in the Fernbach flasks which had been fitted with cotton bungs to allow for ventilation. The autoclaved flasks were allowed to cool, inoculated with *Streptomyces longisporus ruber*, strain M-3 and placed on a rotary or reciprocating shaker until the cultures turned dark red or orange. This usually took from 4 to 5 days. The cultures were then removed and allowed to sit for one to three weeks. During this time large surface colonies usually developed in addition to the spherical colonies present in the media.

Separation of the pigment from the cultures of Streptomyces

The red cell material was separated from the medium either by centrifugation or by passing the cultures through a series of screens of diminishing mesh. The cell material could then be scraped from the screening. The latter method proved to be the more effective of the two. The cell material was homogenized in a blender and lyophilized. The dried cells were extracted with methylene chloride in a Soxhlet extractor. The dark red methylene chloride extract was concentrated and washed with two portions of aq 1 N NaOH. This was followed by a single wash with 1 N HCl. It was necessary to break up troublesome emulsions which formed during these washings by adding a little methanol. The extract was then dried (Na_2SO_4) and concentrated *in vacuo* yielding a dark oil. Petroleum ether was added to the oil and the solution was cooled at −5° for 1–3 days. The solid hydrochloride of the pigment was removed by filtration and washed with cold petroleum ether. The average yield of dried mycelium was 2 g per Fernbach flask. On extraction, 100 g of mycelium would yield between 1 and 6 g of crude pigment, the average being 4 g. Qualitative TLC analysis (silica gel H, 1% methanol in chloroform) revealed that the product, isolated as a hydrochloride salt, was a mixture of two pigments.

Isolation of undecylprodigiosin **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 band was eluted with 10% chloroform. This substance was not an acid–base indicator and was not investigated further. The main band of pigment was eluted with a 30% chloroform resulting in a partial separation of the two pigments. By taking the band off in 50 ml fractions, it was possible to obtain the first pigment, metacycloprodigiosin,[5] in a pure state. The remainder of the band which contained a mixture of metacycloprodigiosin and undecylprodigiosin **1** was washed with 1 N HCl, dried (Na_2SO_4), and concentrated *in vacuo* leaving a dark-red, amorphous solid.

The solid was taken up in hot carbon tetrachloride and the solution cooled to yield 0.1 g of metacycloprodigiosin hydrochloride. The mother liquor from this crystallization contained the hydrochloride of the second pigment and a residual amount of the first. A black, amorphous solid was obtained from the mother liquor by dilution with petroleum ether and then cooling. Qualitative TLC analysis (silica gel H, 1% methanol in chloroform) indicated that the black solid consisted mainly of the hydrochloride of the second pigment. Repeated crystallizations of the black solid from heptane yielded the hydrochloride of undecylprodigiosin **1** as small, red needles which showed dimorphism: m.p. 76–78° and 106–107°; λ_{max} (CH_3OH + 0.5% KOH) 462 nm (ϵ 40,200) 530 sh (7000); λ_{max} (CH_3OH + HCl) 500 nm (sh) (ϵ 50,000), 530 (101,000); IR (KBr) 3150, 3100, 2947, 2915, 2841, 1615, 1556, 1516, 1364, 1347, 1288, 1269, 1250, 1181, 1140, 1041, 990, 959, 788, 779, 748 cm^{-1}; NMR ($CDCl_3$) τ −2.83 (broad, 3 H), 2.78 (m, 1 H), 3.06 (s, 1 H), 3.10 (m, 2 H), 3.67 (m, 1 H), 3.80 (m, 1 H), 3.96 (d, 1 H), 6.05 (s, 3 H), 7.06 (t, 2 H), 8.76 (m, 18 H), 9.13 (t, 3 H); MS *m/e* (rel intensity) 395 (10), 394 (30), 393 (100), 378 (11), 253 (41), 252 (80), 238 (24), 91 (45).

†We thank Dr. Harashima for sending us a sample of his C-25 pigment for this comparison.

N,N'-Dideuteroundecylprodigiosin deuterochloride

A sample of undecylprodigiosin hydrochloride (45 mg) was dissolved in deuterochloroform (350 μl). The solution was transferred to an NMR tube and approximate 1 ml of deuterium oxide was added along with a trace of hydrogen chloride gas. After the tube was shaken vigorously, it was found that all of the N-protons had exchanged as shown by the NMR spectrum ($CDCl_3$): τ 2.78 (dd, 1 H, J = 1.4, 2.3 Hz), 3.04 (s, 1 H), 3.10 (m, 1 H), 3.17 (d, 1 H, J = 3.8 Hz), 3.67 (dd, 1 H, J = 2.3, 3.8 Hz), 3.80 (d, 1 H, J = 3.8 Hz), 3.94 (s, 1 H), 6.01 (s, 3 H), 7.05 (t, 2 H, J = 7.5 Hz), 8.72 (broad singlet, 18 H), 9.11 (t, 3 H).

The zinc derivative of undecylprodigiosin 1

The zinc derivative of undecylprodigiosin 1 was obtained by the previously decribed method.[6] Crystallization of the crude produce from ethanol-water yielded the pure compound: m.p. 102–103°; IR (KBr) 3330, 2950, 2920, 2850, 1610, 1595, 1578(sh), 1539 1475, 1371, 1230, 1179, 1133, 1121, 1106, 1039, 973, 909, 840, 790, 730 cm^{-1}. (Found: C, 70.82; H, 8.10; N, 9.90; Zn, 8.10. Calc. for $(C_{25}H_{34}N_3O)_2Zn$: C, 70.60; H, 8.01; N, 9.89; Zn, 7.70%).

Pyrolysis of undecylprodigiosin 1

A sample of amorphous undecylprodigiosin hydrochloride (115 mg, 0.36 mmol) (shown to be essentially pure by TLC) was intimately ground with soda lime (3 g) which had been dried for 2 h over a Bunsen burner. This mixture was then put in a pyrolysis tube over a glass wool plug and 1" of pure soda lime. The tube was sealed, evacuated to 0.1 mm or less and maintained at reduced pressure for 1 h. At the end of this time, the tube, still under vacuum, was slowly pushed into a tube heater at 400°. When half of the plug of pure soda lime was in the tube heater, the pyrolysis tube was allowed to stand for 10 min (when the pyrolysis tube was not allowed to stand as described, several side products were observed). The entire tube was then inserted into the heater. In a few minutes, partially solid 2-*n*-undecylpyrrole 3 (46 mg, 57%) condensed on the walls of the pyrolysis tubes just outside of the tube heater. This product was shown to be more than 95% pure by GLC. Crystallization from acetone yielded pure 3: m.p. 40–41°; IR (CCl_4) 3478 (sharp), 3385 (broad), 3000, 2955, 2930, 2920, 2850, 1566, 1560, 1466, 1458, 1118, 1090, 1024, 882, 705 cm^{-1}; NMR (CCl_4) τ 2.55 (broad, 1 H), 3.58 (m, 1 H), 4.07 (m, 1 H), 4.24 (m, 1 H), 7.52 (t, 2 H), 8.2–8.9 (broad singlet, 18 H), 9.11 (t, 3 H); MS *m/e* (rel intensity) 221 (46), 80 (100).

Synthesis of 2-n-*undecylpyrrole* 3

The procedure described below is a modification of that used by Skell and Bean to synthesize 2-*sec*-butylpyrrole.[11]

An ethereal solution of ethylmagnesium bromide from magnesium turnings (7 g, 0.3 mol) and ethyl bromide (27.2 g, 0.25 mol) was prepared under nitrogen. The mixture was cooled to 0°, and freshly distilled pyrrole (16.8 g, 0.25 mol) in 50 ml of anhydrous ether was added dropwise to the stirred solution. Evolution of a gas was noted. After addition was complete the reaction mixture was heated at reflux temp for 0.5 h. *n*-Undecyl bromide (59 g, 0.25 mol) in 100 ml of anhydrous ether was then added dropwise and the resultant solution heated at reflux temp for 24 h. The mixture was cooled, and the magnesium salts decomposed with saturated ammonium chloride solution. The layers were separated, and the aqueous layer was extracted with ether. The combined ether layers were washed with water, sat NaCl soln, dried ($MgSO_4$), and decolorized with activated charcoal. The ether was then removed *in vacuo*. Cooling of an acetone solution of the residue yielded 3 g of a solid. Preliminary evidence indicated that the solid consisted of polyalkylated pyrroles. The solvent was removed *in vacuo* from the mother liquor of the crystallization, and the residue was distilled to yield monoalkylated pyrroles (12 g, 22%), b.p. 110°/0.8 mm. GLC analysis (12' × 3/8" 15% Carbowax 20 M on 70/80 mesh Anakrom ABS column, 210°) indicated that both the 2- and 3- isomers were present in about a 9:1 ratio. Crystallization from acetone yielded pure 2-*n*-undecylpyrrole 3: m.p. 42–43°. (Found: C, 81.21; H, 12.09; N, 6.74. Calc. for $C_{15}H_{27}N$: C, 81.45; H, 12.22; N, 6.34%). The IR, NMR and MS of the synthetic pyrrole were found to be identical with those of the natural pyrrole.

Synthesis of undecylprodigiosin 1

A solution consisting of 123 mg (0.55 mmol) 2-*n*-undecylpyrrole 3 in 10 ml of ethanol was added to 50 mg (0.26 mmol) of bipyrrolic precursor 4 dissolved in warm ethanol.[6,13-15] Upon addition of 25 drops of conc. HCl the solution turned deep red. It was allowed to stand at room temperature for 22 h. The reaction mixture was diluted with 150 ml of water and extracted with methylene chloride. Qualitative TLC (silica gel H, 1% methanol in chloroform) indicated that three components were present, one red, one blue and one yellow. The red material was the main component.

The methylene chloride extract was washed with 1 N NaOH and dried (Na_2SO_4). The methylene chloride was removed *in vacuo*, and the residue was taken up in petroleum ether and applied to a column of basic alumina (5 g of Fisher, Brockman Activity 1). The column was developed with 150 ml portions of petroleum ether, petroleum ether/chloroform, increasing the percentage of chloroform by 3% each time.

A yellow band was eluted with pure petroleum ether but was present in such small quantity that it was discarded. The undecylprodigiosin 1 was eluted with 12% chloroform. A purple component was eluted with 1% methanol in chloroform. This was washed with acid to yield a brilliant blue solution. Upon removal of solvent, and recrystallization from carbon tetrachloride, 2 mg of deep, blue-black crystals were obtained. The IR spectrum of this component was identical with that of another prodigiosin-like pigment isolated in these laboratories.[16]

The fraction containing the undecylprodigiosin was washed with 1 N HCl to yield a brilliant red solution. The solution was dried (Na_2SO_4) and the solvent removed *in vacuo* leaving a residue which was shown to be essentially pure undecylprodigiosin hydrochloride (1 · HCl) (73 mg, 66%) by TLC (silica gel H, 1% methanol in chloroform). The hydrochloride was taken up in 5 ml of methylene chloride and the solution divided into two portions, A and B:

Part A. The methylene chloride was removed from this portion and the residue was crystallized five times from heptane to yield 5 mg of tiny red crystals, m.p. 76–77° and 106–107°. The synthetic hydrochloride had the same m.p. (m.m.p. 105–106°) and the same IR spectrum as the natural material.

Part B. The methylene chloride solution was washed with 1 N NaOH. The methylene chloride was then removed and the undecylprodigiosin which remained was used to make a zinc derivative by the known method.[6] The resulting derivative was recrystallized twice from ethanol/water to yield 5 mg of material, m.p. 102–103°. the synthetic zinc derivative gave the same m.p. (m.m.p. 100·5–102°) and the same IR spectrum as the material derived from natural sources.

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