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A NOVEL, CYCLIC, TRIPYRROLE PIGMENT FROM ACTINOMADURA (NOCARDIA) MADURAE

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Recently we reported that 16 strains of Actinomadura (Nocardia) pelletieri and A. madurae produce several prodigiosin-like pigments. The major pigment from A. madurae 953 was identified as nonylprodigiosin (1).

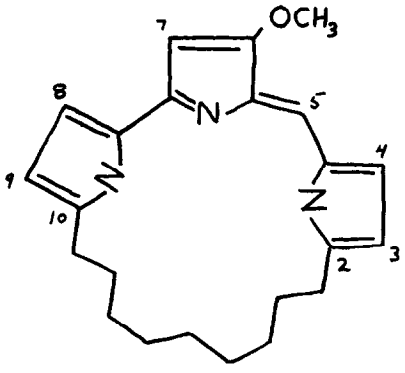
In later fermentations a different pigment, with a higher R_f value on TLC plates, predominated. It was distinct, by direct comparison, from prodigiosin (2), nonyl and undecylprodigiosins (3) and metacycloprodigiosin (4). The new pigment had an intense, yellow, fluorescence on TLC plates in long wave length UV light; the others were a weaker orange. Its perchlorate salt was soluble in hexane and in the 2-phase system hexane-ethanol-acetic acid-water 40:24:1:8, the partition coefficient was ten times that of nonylprodigiosin. The visible absorption spectrum of the new pigment in acidic and basic solvents was similar to that of prodigiosin and the maximum at 542 mμ in acidic ethanol was consistent with dialkyl substitution (not mono) of the aromatic chromophore. Furthermore, the shift in the maximum when the solvent was changed to $CHCl_3$ was +9 mμ (for prodigiosin, nonyl and undecyl-prodigiosin the shift is +3 mμ) suggesting a more rigid, possibly cyclic structure.

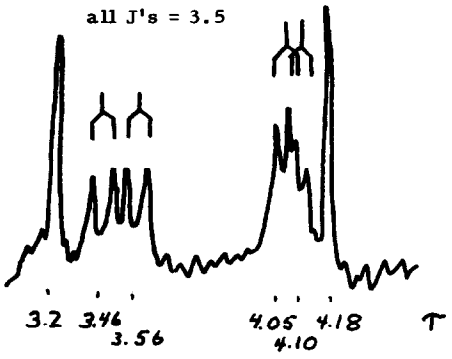
The cyclic structure was verified by the mass spectrum which showed a molecular ion at 363 (100%) and M^{++} at 181.5. The striking feature of the mass spectrum compared to those of undecyl and nonylprodigiosins (1,3) was the significantly greater intensity of the molecular ion peak.

When the NMR spectrum of the new pigment (free base, in $CDCl_3$) was compared with those of others of the prodigiosin group (3, 5, 6) it was possible to assign bands for each proton in the 3-5 τ range. Thus the singlets at 3.2 and 4.2 τ result from the hydrogens at carbons 5 and 7. The remaining bands could be explained as two AB systems, each system containing one "α" and one "β" type of pyrrole proton (5). The methylene band of the new pigment was centered at 8.6 τ whereas in metacycloprodigiosin (which has an 11 carbon side chain attached at positions 2 and 4) this band is at 9.1 τ (6a).

Therefore, in our opinion, the best structure is that shown with the alkyl chain bound at carbons 2 and 10, positions which are also favored by biogenetic considerations. Side chains at carbons 4 and 8 would also give rise to AB systems in the NMR. However, position 8 can be excluded on the basis of the known coupling constants for the protons in the bipyrrole precursor of prodigiosin (11a) which are 1.4 ± 0.2 cps for J(8-10), 3.5 ± 0.2 cps for J(8-9) and 2.6 ± 0.2 cps for J(9-10). All naturally occurring prodigiosin-like pigments have an alkyl substituent at carbon-2 (7). In addition, J(8-9) and J(4-3) in undecylprodigiosin are both 3.8 cps (11b). Thus the observed coupling constant of 3.5 cps is strong evidence for the proposed structure.

In order to confirm the nature of the side chain, the pigments were oxidized with dilute potassium permanganate in aqueous pyridine at 28° with shaking for 2 days. The acids obtained were methylated and analyzed by gas chromatography (8). Nonylprodigiosin yielded methyl nonate and methyl decanoate in a ratio of 1 to 4. The new pigment gave 3 peaks of a homologous series, the dimethyl esters of azelaic, sebacic, and undecanedioic acids, in a 2:7:5 ratio with the same retention times as authentic samples. The mass spectrum of the major product confirmed the unbranched structure since the large peaks due to α cleavage with hydrogen rearrangement were at 74 and 156 (M-74), not 88 as would be expected if there were an α-methyl group (9).





Partial NMR spectrum of cyclic pigment

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7. An apparent exception, Vitamycin A, is reported by Yu. M. Khokhlova, L. N. Sergeeva, N. S. Vul'fson, V. I. Zaretskii, V. G. Zaikin, V. I. Sheichenko and A. S. Khokhlov in Khim. Pric. Soedin, 4, 307 (1968), Chem. Abstr. 70, 87432j (1969). However, the published NMR spectrum of Vitamycin A is identical with that of metacycloprodigiosin (ref. 6a). The published mass spectrum of Vitamycin A is identical with that which we obtained from the C-25 prodigiosin-like pigment of F. Arcamone, A. DiMarco, M. Chione and T. Scotti, Giorn. Microbiol., 4, 77 (1957) which we had earlier shown, by direct comparison, to be identical with metacycloprodigiosin.

8. Using a 6 foot column of 10% SE-30 on Diatoport W 60-80 mesh, in an F & M model 700 dual column instrument with a thermal conductivity detector.

9. J. H. Beynon, R. A. Saunders and A. E. Williams, The Mass Spectra of Organic Molecules, p. 246, 251, Elsevier, 1968. The absence of other aliphatic methyl groups was seen in the NMR spectrum.

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11. (a) L. A. Smith, Ph.D. Thesis, Yale Univ., New Haven, Conn., 1962, p. 59. (b) D. D. Keith, Ph.D. Thesis, Yale Univ., 1969, p. 28. We thank a referee for pointing out this information.