A new red pigment from an alkalophilic Micrococcus species

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From the aqueous acetone extracts of an alkalophilic Micrococcus species, a new tripyrrole red pigment has been isolated. The pigment which has structural similarity to prodigiosins has been characterised as 2,2'-[3-methoxy-1'-amyl-5'-methyl-4'-pyryrlyl]dipyrryl methene on the basis of spectral correlations. Unlike prodigiosins, all the nitrogen atoms are in a tertiary state with the amyl chain attached to nitrogen instead of carbon. This is the first report of such a pigment from Gram positive bacteria. Preliminary studies reveal this pigment to be a potent antioxidant.

Prodigiosins are a class of tripyrrole bright red pigments isolated from several genera of gram negative bacteria such as Serratia, Vibrio, Actinomadura, Pseudomonas and Streptomyces. These compounds exhibit a broad spectrum of biological activity including antileukaemic, antibacterial, antifungal and antimalarial properties. We have been carrying out investigation on the pigments of Micrococcus species isolated from stored dehydrated shrimp. Earlier work on this organism had revealed only the presence of several carotenoid pigments. However, in the present study a deep red pigment was isolated from this organism that had structural similarity to the prodigiosins. We report herein the structure of the new compound as confirmed by spectral analysis.

The pigment showed absorption maxima at 536.1 nm indicative of an extended conjugation in the molecule. The molecular formula of compound 1, C_{20}H_{28}N_{3}O, was derived by a combination of EI/ClI/FAB mass spectrometry (M+, m/z 323) and HREI-mass spectrometry (323.19976). The molecular formula indicates the presence of 10 double bond equivalence that could be accommodated by three pyrrole rings and an ethylenic double bond. The absence of characteristic bands in the IR spectrum for NH groups suggested that all the three nitrogen atoms in the molecule are tertiary in nature. The absence of OH group was also inferred from the IR spectrum, which however indicated the presence of aromaticity in the molecule further suggesting the presence of a pyrrole ring. \(^1\)H NMR spectrum of 1 showed signals for aromatic protons in the region \(\delta 6.1-7.25\) ppm. A group of signals in the high field region \(\delta 0.9-2.35\) indicated the presence of an alkyl side chain further supported by a fragment ion at m/z 266 (M-57) in the mass spectrum. A sharp singlet at \(\delta 5.4\) was attributed to an ethylenic proton while that at 3.99 was assigned to a methoxy functionality. \(^13\)C NMR spectrum of 1 exhibited signals for aromatic carbons in the region \(\delta 110-130\) ppm further supporting presence of pyrrole rings. The presence of a 5 carbon N-alkyl side chain was indicated by appearance of signals between \(\delta 11.5-39.0\). A downfield shift of C-7' in the NMR spectrum (\(\delta H=2.35\) and \(\delta ^{13}C=39\)) indicated attachment of the chain to N group of pyrrole ring. The ethylenic carbon resonated at \(\delta 94.0\). The mass spectrum contained prominent fragments at 323 (M* base) 266 (83.2%), 250 (42%), 162 (2.7%), 133 (7.6%) and 91 (13.1%) clearly suggesting a structural similarity to prodigiosin.

From the foregoing data as well as by comparison of its spectral data with that of prodigiosin structure of the pigment was assigned as 2,2'-(3-methoxy-1'amyl-5'methyl-4'-pyryrlyl)dipyrryl methene.

The pigment was found to be a natural potent antioxidant as shown by carotene bleaching assay. A systematic evaluation of biological activities of the compound is currently in progress.

Experimental Section

General. UV spectra was recorded in MeOH on Varian DMS 100 spectrophotometer (\(\lambda_{max}\) in nm). \(^1\)H NMR (300 MHz) and \(^13\)C NMR (75 MHz) in CDCl$_3$
was carried out on a Brucker AM 200 using TMS as an internal reference (chemical shift in δ, ppm). IR was done on an Impact 410, Nicolet (νmax in cm⁻¹) with spectra referenced to residual solvent signals. EIMS and HREIMS (probe): 70 eV, CIMS (+ve ion) and FABMS (thioglycerol). TLC was performed on silica gel G (type 60) while column chromatography on silica gel (60-120 mesh).

**Extraction and isolation.** Freeze-dried cells of Micrococcus sp. (10 g) were extracted with Me₂CO (3×200 mL) at room temperature. To the extract was added equal amount of water and the mixture repeatedly extracted with Et₂O (4×100 mL). The Et₂O extracts were pooled washed (H₂O) and then dried overnight (Na₂SO₄). Removal of solvent in vacuo yielded a crude oily red pigment that was subjected to CC (50 g x 1.5 cm id x 50 cm) using petrol followed by increasing proportions of Et₂O. Fractions eluting with petrol-Et₂O, 60:40 and 50:50 containing the pigments of interest were pooled and evaporated to dryness in vacuo to obtain partially purified pigment. This was further purified by prep. TLC using petrol-Me₂CO as developing solvent system. The orange-red band at Rf 0.47 was scraped and eluted with MeOH. Solvent was removed as above to obtain the purified pigment that gave as single spot on TLC in solvent systems of varying polarity.

**Compound 1:** Oily (50 mg); IR (CHCl₃): 3018, 2958, 1614, 1580, 1422, 1378, 1273, 1215, 1142, 1072, 757, 668; UV (MeOH): 536.1 nm; 'H NMR (CDCl₃): δ 0.9(t, 3H, H-1'), 1.25(m, 2H, H-10'), 1.27(m, 2H, H-9'), 1.6(m, 2H, H-8'), 2.35(br, 2H, H-7'), 2.45(s, 3H, H-6'), 3.99(s, 3H, H-6), 5.4(s, 1H, H-7), 6.05(s, 1H, H-3'), 6.25(s, 1H, H-4'), 6.5(s, 1H, H-3'), 6.75(s, 1H, H-4'), 6.8(s, 1H, H-2') 7.1(s, 1H, H-5'), 7.25(s, 1H, H-5); ¹³C NMR (CDCl₃): δ 11.5(C-11'), 13(C-6'), 22(C-10'), 25(C-9'), 32(C-8'), 39(C-7'), 59(C-6), 94(C-7), 110(C-3',C-4'), 112(C-3',C-4'), 118(C-5',C-2'), 125(C-5), 126(C-2', 127(C-5',C-2'), 129(C-4'), 131(C-3); HREI-MS m/z (rel. int.): 323.19976[M+] (Ca for C₂₀H₂₅N₃O), m/z 323.1998 (100), 266.1293 (C₁₀H₁₆N₃O) (83.2), 251.0624 (C₁₅H₂₁N₄O) (2.5), 162.0680 (C₁₁H₁₄N₂O) (3), 133.9445 (C₈H₁₁N) (7), 91.000 (C₆H₆N) (17); EIMS m/z (rel. int.): 323(100), 266(80.2), 250 (4.2), 162 (2.7), 133 (7.6), 91 (13.1); CIMS m/z (rel. int.): 323 (100), 266(13), 175(7.7), 162(10.4), 91(3.8), 85(7.7), 69(11.7); FABMS m/z (rel. int.): 324 (100), 323 (19.4), 266 (9.7), 252 (12.6), 162 (4.7), 161 (6.1), 149 (9.1), 133 (9.6), 119 (10.8), 117 (7.9), 109 (12.4), 104 (13.8).

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**References**