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A Blue Pigment from a Compound Ascidian

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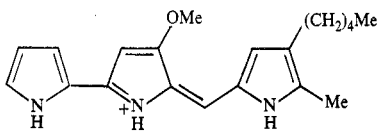
Abstract

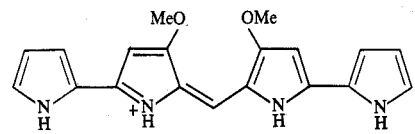
A deep blue pigment, isolated from a Western Australian compound ascidian, has been shown to be identical to a tetrapyrrole reported from a mutant strain of a bacterium.

During a biological survey of the Abrolhos Islands, Western Australia, several specimens of a dark blue compound ascidian were collected. Extraction of freeze-dried material with dichloromethane followed by preparative layer chromatography gave, as the major component, an intensely dark blue pigment which turned red in basic solutions.

The ^{1}H n.m.r. spectrum of this compound indicated the presence of two methoxyl groups (δ 3·94) and a single olefinic proton (δ 5·34). The general symmetry of this spectrum together with a molecular formula of $C_{19}H_{18}N_4O_2$ based on high-resolution mass measurements pointed to the pigment consisting of two C_9 units joined by a methine carbon. This formulation was supported by the ^{13}C n.m.r. spectrum which showed the two C9 units to be very similar. The u.v. spectrum [λ_{max} (MeOH) 591, 555, 325, 280 nm (logε 4·81, 4·46, 4·00, 3·83); λ_{max} (MeOH+OH$^-$) 571, 531, 310, 273 nm (logε 4·79, 4·37, 4·37, 4·43)] was highly unusual and was similar to that found for prodigiosin.[1] Thus a structure consisting of two methoxybipyrrole units joined by a bridging carbon was envisaged and a base peak at *m/e* 163 ($C_9H_{11}N_2O$) in the chemical ionization mass spectrum supported this.



(1)



(2)

Prodigiosin (1) had been isolated from the bacterium *Serratia marcescens*[1] and, subsequently, a purple pigment (2) was observed in mutant strains of the same

[1] Wasserman, H. H., McKeon, J. E., Smith, L. A., and Forgione, P., *Tetrahedron*, Suppl. 8, 1966, 647, and references therein.

organism.[2] The structure of the purple pigment (2), isolated as its hydrochloride, was confirmed by synthesis.[3] The spectral data reported for compound (2), although incomplete, agreed very closely with that of our ascidian pigment but microanalysis demonstrated that the tetrapyrrole (2) isolated from the ascidian contained both chloride and bromide counter-ions. The ease with which the protonated form of (2) could be purified by chromatography on silica gel with relatively non-polar solvents was somewhat surprising.

The small quantity of the pigment isolated precluded extensive pharmacological testing and allowed only *in vitro* screening to be carried out. In the cardiovascular laboratory, screening of the pigment on the electrically driven isolated guinea-pig ileum gave a dose-dependent increase in the contractile force of the atrium and this is shown in Fig. 1.

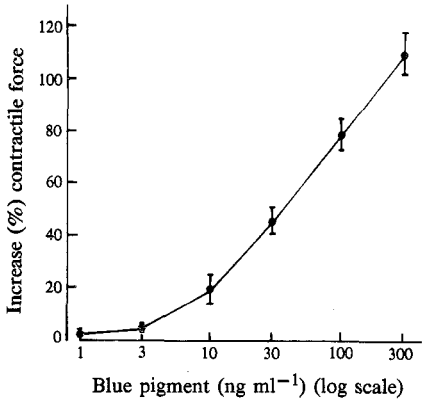


Fig. 1. Biological activity of the pigment.

Experimental

Instruments used to acquire spectral data have been listed elsewhere.[4]

Isolation of the Pigment

Three specimens of the ascidian (5 g dry wt) were exhaustively extracted with dichloromethane (1 l.) to give on evaporation of the solvent a dark blue oil (200 mg). This material was purified by preparative t.l.c. developed in ethyl acetate. The single blue band was eluted with ethyl acetate and gave on evaporation a blue-black solid (40 mg). This was recrystallized from dichloromethane/light petroleum to give blue crystals (20 mg), m.p. >300° (dec.). I.r. spectrum: (KBr) ν_{max} 1635, 1630, 1590, 1537, 1510, 1260, 1233, 960 cm^{-1}. {^{1}H}^{13}C n.m.r. spectrum: 163·0 (s, 2C), 142·7 (s, 2C), 124·2 (d), 124·0 (d), 122·8 (s), 122·7 (s), 117·1 (s), 117·0 (s), 113·9 (d, 2C), 110·9 (d, 2C), 109·0 (d), 92·2 (d, 2C), 58·3 (q, 2C) ppm. Mass spectrum: (e.i.) *m/e* 335 (40%), 334 (100), 320 (18), 319 (40), 288 (13), 200 (20), 167 (14), 162 (20), 92 (26), 91 (53). High-resolution mass measurement: Found *m/e* 334·1411, 319·1180, 288·0996; $C_{19}H_{18}N_4O_2$, $C_{18}H_{15}N_4O_2$, $C_{17}H_{12}N_4O$ require mol. wts, 334·1428, 319·1193 and 288·1009 respectively. Mass spectrum: (c.i. isobutane) 336 (20%), 335 (54), 334 (75), 219 (15), 205 (18), 175 (39), 163 (100), 162 (75). High-resolution mass measurement: Found *m/e* 334·1418, 335·1453, 163·0864, 162·0790; $C_{19}H_{18}N_4O_2$, $C_{19}H_{19}N_4O_2$, $C_9H_{11}N_2O$, $C_9H_{10}N_2O$ require mol. wts, 334·1428, 335·1507, 163·0870, 162·0792 respectively. ^{1}H n.m.r. spectrum: δ ($CDCl_3$) 7·16, 2H, s; 6·86, 2H, m (D_2O exch, dd, *J* 4, 1 Hz); 6·40, 2H, m (D_2O exch, dd, *J* 4, 2·5 Hz); 6·10, 2H, d, *J* 2·5 Hz (D_2O exch, s); 5·34, 1H, s; 3·94, 6H, s.

[2] Janes, D. W., Goldschmidt, M. E., Cash, H. P., and Williams, R. P., *Tex. Rep. Biol. Med.*, 1966, **24**, 489.

[3] Wasserman, H. H., Friedland, D. J., and Morrison, D. A., *Tetrahedron Lett.*, 1968, 641.

[4] Kazlauskas, R., Marwood, J. F., and Wells, R. J., *Aust. J. Chem.*, 1980, **33**, 1799.

Acknowledgments

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