Bioactive marine metabolites. VIII. Isolation of an antimicrobial blue pigment from the bryozoan *Bugula dentata*[1]

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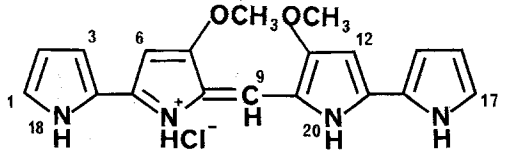
Summary. An antimicrobial blue pigment, which is identical with a tetrapyrrole from a bacterium, was isolated from the bryozoan *Bugula dentata.*

Key words. Bugula dentata; bryozoa; antimicrobial; tetrapyrrole.

In spite of their wide distribution in tropical and temperate waters[2], marine bryozoans have until recently attracted little attention from marine natural product chemists[3]. However, several novel compounds have recently been isolated from them; physostigmine alkaloids[4-7], methyltryptamine derivatives[8,9], and a bromoquinoline[10] from the cold water species *Flustra foliacea;* (2-hydroxyethyl)dimethylsulfoxonium ion from the Dogger Bank itch-causative organism *Alcyonidium gelatinosum*[11]; and the antineoplastic macrolides, bryostatins, from the cosmopolitan species *Bugula neritina*[12-15]. In the course of our search for biologically active substances from Japanese marine invertebrates, we have found that a common bryozoan, *Bugula dentata* Ramouroux, possesses a brilliant blue pigment, which is antimicrobial against gram-positive and gram-negative bacteria. We have isolated the pigment, which we deduced by spectral means to be identical with a tetrapyrrole isolated from a mutant strain of *Serratia marcescens.*

The ethanol extract of the frozen specimens (550 g) of *Bugula dentata* collected in the Gulf of Sagami, Japan, was partitioned between water and diethyl ether. The ether soluble material (4.4 g) was chromatographed on a silica gel column (5.5 × 30 cm) with benzene-ethyl acetate (85:15), and subsequently on a Sephadex LH-20 column (5.5 × 30 cm) with hexane-chloroform-methanol (2:1:1) to yield a blue pigment (120 mg). This showed in vitro antimicrobial activity against *E. coli, S. aureus,* and *B. subtilis.* The ^1H and the ^{13}C NMR spectra revealed the presence of two monosubstituted pyrroles [δ 6.31 (2H, ddd, J = 2.5, 2.5, 3.7 Hz, H-2,16), 6.79 (2H, ddd, J = 1.3, 2.5, 3.7 Hz, H-3,15), 7.12 (2H, m, H-1,17), 11.77 (2H, m, H-18,21); δ 123.5 s (C-4,14), 114.5 d ($^1J_{C-H}$ = 173.0 Hz, C-3,15), 111.6 d (172.9 Hz, C-2,16), 124.8 d (187.5 Hz, C-1,17)], two methoxy pyrroles [δ 3.94 (6H, s, OCH_3), 6.06 (2H, d, J = 2.0 Hz, H-6,12), 11.97 (2H, m, H-19,20); δ 58.9 q (145.0 Hz, OCH_3); 117.1 s (C-8,10), 163.7 s (C-7,11), 92.9 d (175.8 Hz, C-6,12), 143.3 s (C-5,13)], and a vinyl group [δ 7.12 (1H, s, H-9); δ 109.8 d (156.8 Hz, C-9)]. The integration ratio of 1:2 for the vinylic proton and others suggested the molecule to be symmetrical, which was supported by the high resolution mass spectrum (m/z 334.1482; $C_{19}H_{18}N_4O_2$, calculated for 334.1430). The UV-VIS spectrum [λ_{max} ($CHCl_3$) 599.0 (ε 103,000), 556.0 (28,400), 326.0 (26,300)] was also characteristic of compounds of the prodigiosin type[16]. These data were compatible with the tetrapyrrole pigment **1** isolated from a mutant strain of the bacterium *Serratia marcescens*[16]. The spectral data except for the chemical shift of the vinylic proton, which is not reported, were consistent with those of our pigment. Recently Kazlauskas et al.[17] reported the isolation of the same pigment from an Australian ascidian. Their spectral data coincide well with ours except for the ^1H NMR chemical shift of the C-9 vinylic proton (δ 7.12 vs 5.34). This difference may be due to a difference either in the counter ion, the geometry of the C-8,9 double bond[18], or misassignment. Our value is in good agreement with those of prodigiosin (δ 6.97)[19] and cycloprodigiosin (δ 7.04)[20]. Microanalysis demonstrated that the tetrapyrrole isolated from the bryozoan contained chloride as the counter-ion. Therefore, we conclude that our pigment possesses the structure **1**.



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A number of bacteria, including marine bacteria, have been shown to produce prodigiosin-like pigments[19-21]. The color of the bryozoan *B. dentata* is usually dark blue, which suggests that pigment **1** is ubiquitously present in the animal. Whether the compound is biosynthesized by the bryozoan itself or by an associated microorganism or derived from food sources such as prodigiosin-producing bacteria is unknown.

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