Biosynthetic Studies of the Alkaloid, Stevensine, in a Cell Culture of the Marine Sponge *Teichaxinella morchella*

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Abstract: The biosynthetic origin of stevensine, a $C_{11}N_5$ sponge alkaloid, has been investigated using a cell culture composed of archaeocytes from the sponge *Teichaxinella morchella* (Porifera: Demospongiae: Axinellidae). Histidine and ornithine/proline were identified as amino acid building blocks of stevensine.

Marine sponges belonging to various genera have yielded structurally intriguing and biologically active metabolites belonging to the "oroidin" (**1**) class of marine alkaloids.[1,2] Stevensine (**2**) is representative of a family of tricyclic alkaloids within the oroidin class possessing a pyrrolo[2,3-*c*]azepin-8-one fused bicyclic system unique to these marine alkaloids. Stevensine (**2**), originally isolated and characterized from an unidentified Micronesian sponge[3] and the New Caledonian sponge, *Pseudaxinyssa cantharella* (Axinellidae),[4] has been identified as a major secondary metabolite of the Caribbean sponges *Teichaxinella morchella* Wiedenmayer (Axinellidae) (=*Axinella corrugata*[5]) and *Ptilocaulis walpersi* Duchassaing and Micholotti (Axinellidae).[6] Recent efforts to synthesize **2** and related natural products have been realized,[7-9] however, there have been no reported investigations into the biosynthesis of **2** or of any of its analogues. In this communication, we describe the results of a biosynthetic study using a cell culture of *T. morchella*.

The use of cell cultures for biosynthetic investigations has been successful with plants and microorganisms, however, the difficulty of establishing continuous cell lines of marine invertebrates has prohibited the use of cell culture as a means of addressing biosynthetic origins of marine invertebrate-derived natural products. Our group at Harbor Branch Oceanographic Institution has established primary cultures of *T. morchella* archaeocytes, which retain the ability to synthesize **2** after doubling.[10] To our knowledge, this represents the first biosynthetic study of a natural product from a marine invertebrate utilizing a cell culture of the source organism.

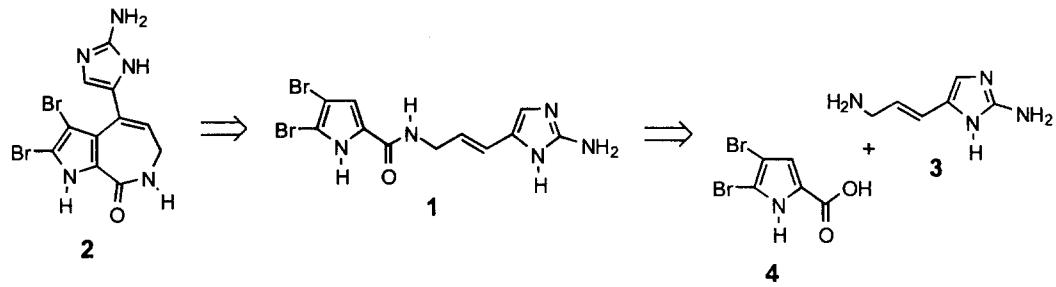

Figure 1. Biosynthetic analysis of stevensine.

Presumably, stevensine (**2**) is derived from a cyclization of oroidin (**1**) which appears to be the condensation product of 3-amino-1-(2-aminoimidazolyl)-prop-1-ene (**3**) and 4,5-dibromopyrrole carboxylic acid (**4**) (Figure 1). This route is supported by the reported co-occurrence of **3** and **4** with **2**, in *T. morchella*.[6]

We postulate that **3** could be derived from either histidine (**5**) or arginine (**6**). Figure 2 outlines potential biosynthetic routes leading from **5** and **6** to intermediate **3**. In the first pathway, **5** undergoes an oxidative deamination, followed by reduction of the carboxyl with subsequent amination to afford intermediate **7**. Introduction of an additional amino group on the imidazole moiety would yield **3**. Alternatively, **6** could be transformed into the aminoimidazole **3** by methylation of the guanidinium functionality followed by ring closure and decarboxylation of **8**.

A biosynthetic analysis of **4** also suggests the possibility of two different amino acid precursors. As indicated in Figure 3, both ornithine (**9**) and proline (**10**) could be transformed to an intermediate such as **11**, which upon bromination could yield **4**.

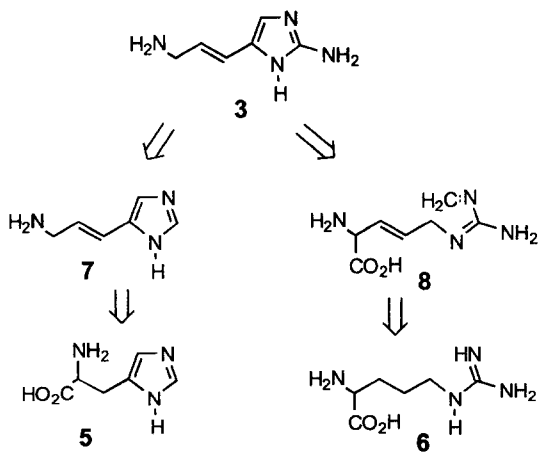

Figure 2. Biosynthetic analysis of **3**.

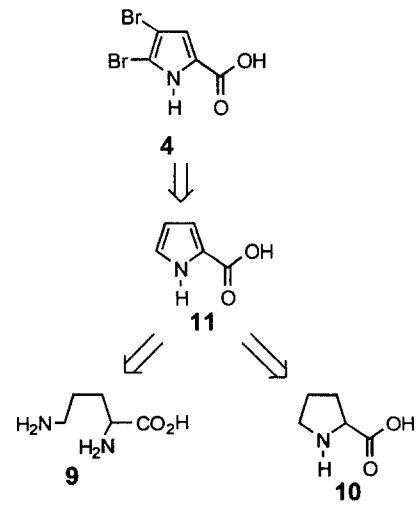

Figure 3. Biosynthetic analysis of **4**.

Four identical cultures, each consisting of 50 million viable *T. morchella* archaeocyte cells, were prepared according to previously reported methodology.[10] The small amount of biological material available in the cell culture system necessitated the use of radiolabeled amino acids to effectively detect incorporation of the precursors into **2**. Individual ^{14}C-labeled amino acids (**5**, **6**, **9**, **10**) were added to the culture medium and the cells maintained at room temperature for 48 hr. The entire cultures (cells and medium) were then lyophilized, extracted with methanol, and concentrated. An aliquot (50%) of the methanolic extract was subjected to reversed-phase (C-18) flash chromatography using a step gradient of 100% H_2O (0.1% TFA) to 100% CH_3CN in 5 steps. A portion (25%) of the 60:40 eluate was further purified by reversed-phase HPLC (Altex Ultrasphere-ODS) equipped with a diode array UV detector using an authentic sample of **2** as cold carrier. As described in Table 1, ^{14}C-histidine, ^{14}C-ornithine and ^{14}C-proline gave radioactive stevensine. Radiochemical purity was established for all cases where significant radioactivity was found to be associated with the stevensine HPLC peak. Firstly, fractions prior to, and following, the stevensine peak were analyzed for radioactivity and in each instance found to not be radioactive. Further, a second portion (25%) of the 60:40 (H_2O (0.1% TFA): CH_3CN) fraction from the flash chromatography was subjected to HPLC analysis using a different column (Vydac Protein and Peptide C-18) and different eluting conditions. This second analysis gave comparable results with that of the first HPLC analysis reported in Table 1.

Table 1. Recovered radioactivity in stevensine (**2**)

Labeled amino acid	Recovered radioactivity in **2**	% Incorporation in **2**
[U-^{14}C]histidine (5.5×10^6 dpm)	1460 dpm	.026
[U-^{14}C]arginine (5.5×10^6 dpm)	background	----
[C_5-^{14}C]ornithine (5.5×10^6 dpm)	1300 dpm	.024
[U-^{14}C]proline (5.5×10^6 dpm)	1180 dpm	.022
control[11]	background	----

The results indicate that histidine, and not arginine, is a precursor involved in stevensine biosynthesis. Interestingly, both ornithine and proline were transformed to the biosynthetic target,[12] presumably via an intermediate such as **11**.

Further experiments with the cell culture system are currently being investigated to fully elucidate the biosynthetic origin of stevensine. The cell culture of *T. morchella* provides a convenient system for investigating the biosynthetic origin of stevensine and oroidin and serves as a novel *modus operandi* for the exploration of the biosynthesis of marine natural products.

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11. A control experiment was performed by incubating 0.5 μCi (1.1×10^6 dpm) of each amino acid in 10 ml of cell culture medium. The radioactivity recovered from the corresponding flash column fraction was comparable to background, indicating that the sponge cells were responsible for the production of radiolabeled stevensine.

12. It is conceivable that ornithine is transformed into **2** via proline; this will be investigated in our continuing studies.