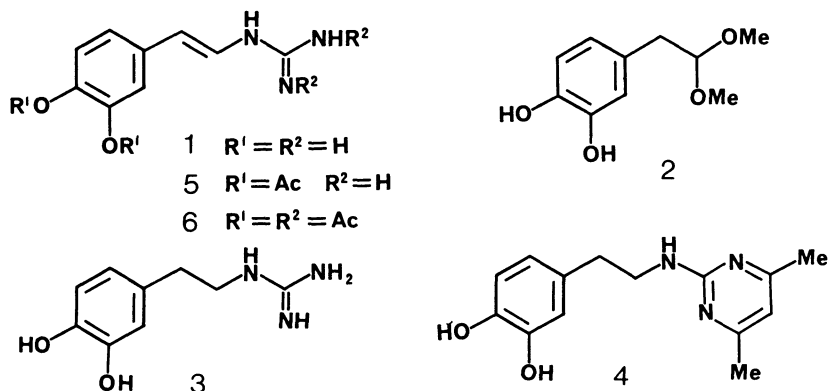


Tubastrine, a New Guanidinostyrene from the Coral *Tubastrea aurea*Ryuichi SAKAI and Tatsuo HIGA\*<sup>†</sup>Harbor Branch Oceanographic Institution-SeaPharm Research Laboratories,  
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Tubastrine has been isolated as an antiviral constituent of the coral *Tubastrea aurea*, and its structure was shown to be  $\beta$ -(amino-iminomethyl)amino-3,4-dihydroxystyrene.

A bright, orange-red coral, *Tubastrea aurea* is a conspicuous organism in rather shady zones of the coral reefs of Okinawa. A polar extract of this species exhibited mild antiviral activity against herpes simplex virus type 1 and vesicular stomatitis virus. In this paper we report the isolation and structure of an active constituent, tubastrine ( $\lambda$ ).

A fresh sample (2 kg), collected at a reef at Onna, Okinawa in April, 1985, was extracted with acetone. After concentration the extract was partitioned between ethyl acetate and water. The aqueous layer was freeze-dried to give a solid residue. Methanol soluble portion (18 g) of the residue showing antiviral activity was subjected to chromatography successively on polystyrene (MeOH-water 1:1, then MeOH), silica gel (*i*PrOH-EtOAc-water 5:4:1), TSK HW-40 (MeOH), and on Sephadex LH-20 (MeOH) to give 1.1 g of tubastrine ( $\lambda$ )<sup>1)</sup> as light yellow solid, mp 173-175 °C. The molecular formula C<sub>9</sub>H<sub>11</sub>N<sub>3</sub>O<sub>2</sub> was deduced from low resolution FABMS (M<sup>+</sup>+1, m/z 194) and <sup>13</sup>C NMR data [ $\delta$  (CD<sub>3</sub>OD) 155.9s, 146.5s, 146.2s, 128.7s, 120.0d, 119.2d, 118.5d, 116.5d, and 113.7d]. The <sup>1</sup>H NMR spectrum (C<sub>5</sub>D<sub>5</sub>N) showed signals [ $\delta$  7.30 (1H, d, J=1.9 Hz), 7.10 (1H, d, J=8.1 Hz), 6.80 (1H, dd, J=8.1, 1.9 Hz), 7.50 (1H, d, J=14.0 Hz), and 6.67 (1H, d, J=14.0 Hz)] indicative of  $\beta$ ,3,4-tri-substituted styrene. The remaining signal was a broad peak at  $\delta$  9.22; suggesting N-bound protons. The presence of a guanidino group was implied by positive Sakaguchi test and by the <sup>13</sup>C NMR signal at  $\delta$  155.9. However,  $\lambda$  did not react with pentane-2,4-



dione to give a pyrimidine derivative.<sup>2)</sup> Treatment of **1** with 2N HCl in methanol under reflux afforded acetal **2** as identified by spectroscopic data.<sup>3)</sup> Hydrogenation of **1** over Pd/C furnished dihydrotubastrine (**3**)<sup>4)</sup> which could now be successfully condensed with pentane-2,4-dione to give pyrimidine **4**,<sup>5)</sup> thus confirming the guanidino function. These results are consistent with the structure of tubastrine being  $\beta$ -(aminoiminomethyl)amino-3,4-dihydroxystyrene (**1**). Although  $\beta$ -aminostyrene functionality is not without precedent in marine metabolites, as seen in such novel compounds as celenamides from a sponge, tunichrome B-1 from a tunicate, and amathamides from a bryozoan,<sup>6)</sup> tubastrine (**1**) is a first example having a simple combination of a guanidine and a dihydroxystyrene and exhibiting antiviral activity.<sup>7)</sup>

## References

- 1**: UV (MeOH)  $\lambda_{\text{max}}$  222 ( $\epsilon$  14500), 280 sh (17400), 287 (18500), and 304 nm (14000); IR (KBr) 3320, 3160, 1670, 1640, 1600, 1520, 1440, 1350, 1300, 1275, 1190, 1155, 1110, and 930  $\text{cm}^{-1}$ . Acetylation ( $\text{Ac}_2\text{O}/\text{C}_5\text{H}_5\text{N}$ , room temp) of **1** for 25 min gave diacetate **5**, while for 12 h tetraacetate **6**. **5**: Mp 185-192 °C;  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  7.27 (1H, d,  $J=8.3$  Hz), 7.26 (1H, s), 7.21 (1H, d,  $J=13.9$  Hz), 7.13 (1H, d,  $J=8.3$  Hz), 6.27 (1H, d,  $J=13.9$  Hz), 2.26 (3H, s), 2.25 (3H, s); LR-FABMS  $m/z$  278 ( $\text{M}^++1$ ), 236, 194, 135, 119, and 103. **6**: Mp 148-150 °C;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  13.14 (1H, br s), 10.77 (1H, d,  $J=10.4$  Hz), 7.65 (1H, dd,  $J=14.8$ , 10.4 Hz), 7.22 (1H, dd,  $J=8.3$ , 2.0 Hz), 7.17 (1H, d,  $J=2.0$  Hz), 7.12 (1H, d,  $J=8.3$  Hz), 6.17 (1H, d,  $J=14.8$  Hz), 2.30 (3H, s), 2.29 (3H, s), 2.23 (3H, s), and 2.21 (3H, s);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  186.3s, 172.7s, 168.2s, 151.9s, 142.3s, 141.9s, 134.8s, 124.1d, 123.6d, 122.9d, 120.4d, 114.4d, 28.7q, 25.1q, 20.6qx2.
- G. T. Carter and K. L. Rinehart, Jr., *J. Am. Chem. Soc.*, **100**, 4302 (1978).
- 2**: Mp 94-97 °C;  $^1\text{H}$  NMR (acetone- $d_6$ )  $\delta$  6.72 (1H, d,  $J=2.0$  Hz), 6.70 (1H, d,  $J=8.0$  Hz), 6.55 (1H, dd,  $J=8.0$ , 2.0 Hz), 4.43 (1H, t,  $J=5.8$  Hz), 3.25 (6H, s), and 2.69 (2H, d,  $J=5.8$  Hz);  $^{13}\text{C}$  NMR (acetone- $d_6$ )  $\delta$  145.3s, 144.0s, 129.6s, 121.3d, 117.1d, 115.5d, 106.2d, 53.0 qx2, and 39.4t. Diacetate of **2**: Oil;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.10 (2H, m), 7.07 (1H, d,  $J=1.7$  Hz), 4.50 (1H, t,  $J=5.5$  Hz), 3.33 (6H, s), and 2.89 (2H, d,  $J=5.5$  Hz); EIMS  $m/z$  282 ( $\text{M}^+$ , 4.6 rel%), 251 (32), 209 (54), 167 (43), 135 (20), 123 (44), and 75 (100).
- 3**: Mp 152-157 °C;  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  6.65 (1H, d,  $J=7.8$  Hz), 6.63 (1H, br s), 6.50 (1H, d,  $J=7.8$  Hz), 3.31 (2H, t,  $J=6.6$  Hz), and 2.65 (2H, t,  $J=6.6$  Hz);  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  158.6s, 146.4s, 145.1s, 130.7s, 121.1d, 116.9d, 116.6d, 44.0t, and 35.3t.
- 4**: Mp 164 °C;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  6.64 (1H, d,  $J=2$  Hz), 6.57 (1H, d,  $J=8$  Hz), 6.48 (1H, dd,  $J=8$ , 2 Hz), 6.32 (1H, s), 5.17 (br s, OH), 3.62 (2H, q,  $J=5.7$  Hz), 2.74 (2H, t,  $J=6.2$  Hz), and 2.29 (6H, s);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  167.7s, 161.3 sx2, 144.7s, 143.1s, 130.8s, 120.5d, 115.3d, 114.9d, 109.8d, 42.3t, 34.5t, and 23.5 qx2; HR-EIMS  $m/z$  259.1323 (calcd for  $\text{C}_{14}\text{H}_{17}\text{N}_3\text{O}_2$  259.1321).
- R. J. Stonard and R. J. Andersen, *J. Org. Chem.*, **45**, 3687 (1980); R. J. Stonard and R. J. Andersen, *Can. J. Chem.*, **58**, 2121 (1980); R. C. Bruening, E. M. Oltz, J. Furukawa, K. Nakanishi, and K. Kustin, *J. Am. Chem. Soc.*, **107**, 5298 (1985); A. J. Blackman and D. J. Mathews, *Heterocycles*, **23**, 2829 (1985).
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