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Tubastrine, a New Guanidinostyrene from the Coral *Tubastrea aurea*

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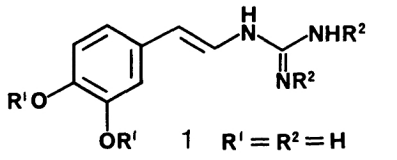
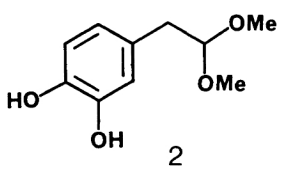
Tubastrine has been isolated as an antiviral constituent of the coral *Tubastrea aurea*, and its structure was shown to be β-(amino-iminomethyl)amino-3,4-dihydroxystyrene.

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 (1).

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 (1)[1] as light yellow solid, mp 173-175 °C. The molecular formula $C_9H_{11}N_3O_2$ was deduced from low resolution FABMS (M^++1, m/z 194) and ^{13}C NMR data [δ (CD_3OD) 155.9s, 146.5s, 146.2s, 128.7s, 120.0d, 119.2d, 118.5d, 116.5d, and 113.7d]. The ^1H NMR spectrum (C_5D_5N) showed signals [δ 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 β,3,4-tri-substituted styrene. The remaining signal was a broad peak at δ 9.22, suggesting N-bound protons. The presence of a guanidino group was implied by positive Sakaguchi test and by the ^{13}C NMR signal at δ 155.9. However, 1 did not react with pentane-2,4-



1 $R^1=R^2=H$
5 $R^1=Ac$ $R^2=H$
6 $R^1=R^2=Ac$



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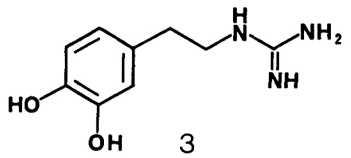

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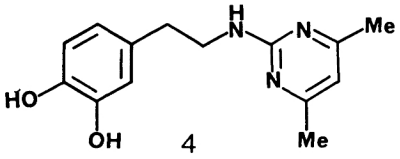

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dione to give a pyrimidine derivative.[2] Treatment of 1 with 2N HCl in methanol under reflux afforded acetal 2 as identified by spectroscopic data.[3] Hydrogenation of 1 over Pd/C furnished dihydrotubastrine (3)[4] which could now be successfully condensed with pentane-2,4-dione to give pyrimidine 4,[5] thus confirming the guanidino function. These results are consistent with the structure of tubastrine being β-(aminoiminomethyl)amino-3,4-dihydroxystyrene (1). Although β-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,[6] tubastrine (1) is a first example having a simple combination of a guanidine and a dihydroxystyrene and exhibiting antiviral activity.[7]

References

1) 1: UV (MeOH) λ_{max} 222 (ε 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 cm^{-1}. Acetylation (Ac_2O/C_5H_5N, room temp) of 1 for 25 min gave diacetate 5, while for 12 h tetraacetate 6. 5: Mp 185-192 °C; ^1H NMR (CD_3OD) δ 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 (M^++1), 236, 194, 135, 119, and 103. 6: Mp 148-150 °C; ^1H NMR ($CDCl_3$) δ 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}C NMR ($CDCl_3$) δ 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.

2) G. T. Carter and K. L. Rinehart, Jr., J. Am. Chem. Soc., 100, 4302 (1978).

3) 2: Mp 94-97 °C; ^1H NMR (acetone-d_6) δ 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}C NMR (acetone-d_6) δ 145.3s, 144.0s, 129.6s, 121.3d, 117.1d, 115.5d, 106.2d, 53.0 qx2, and 39.4t. Diacetate of 2: Oil; ^1H NMR ($CDCl_3$) δ 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 (M^+, 4.6 rel%), 251 (32), 209 (54), 167 (43), 135 (20), 123 (44), and 75 (100).

4) 3: Mp 152-157 °C; ^1H NMR (CD_3OD) δ 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}C NMR (CD_3OD) δ 158.6s, 146.4s, 145.1s, 130.7s, 121.1d, 116.9d, 116.6d, 44.0t, and 35.3t.

5) 4: Mp 164 °C; ^1H NMR ($CDCl_3$) δ 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}C NMR ($CDCl_3$) δ 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 $C_{14}H_{17}N_3O_2$ 259.1321).

6) 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).

7) We thank Dr. Sue Cross for antiviral test.

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