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Narains: *N, N*-Dimethylguanidinium Styryl Sulfates, Metamorphosis Inducers of Ascidian Larvae from a Marine Sponge *Jaspis* sp.

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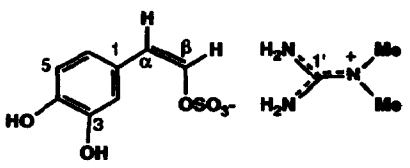
Abstract: Two *N, N*-dimethylguamidinium styryl sulfates, (*E*)-and (*Z*)-narains, which induce metamorphosis of the ascidian *Halocynthia roretzi* larvae have been isolated from a marine sponge *Jaspis* sp. The structures were determined by spectral analysis.

In the course of our studies on inducers/promoters of larval settlement and metamorphosis we have isolated urochordamines A and B[1a] from two ascidians *Ciona savignyi* and *Botrylloides* sp., and phlorotannins and sulfoquinovosyl diacylglycerols[1c] from the brown alga *Sarggassum thunbergii* which promoted larval metamorphosis in two ascidians *Halocynthia roretzi* and *C. savignyi*. Subsequently, we discovered similar activity in the hydrophilic extract of a marine sponge, *Jaspis* sp, collected off the Izu Peninsula. Bioassay-guided isolation afforded two active metabolites, (*E*)- and (*Z*)-narains.[2] This paper describes the isolation and structural elucidation of these compounds.

The wet sponge was extracted with MeOH; the concentrated residue was partitioned against Et_2O, followed by *n*-BuOH. The *n*-BuOH layer, which showed metamorphosis-inducing activity in the tadpole larvae of *H. roretzi*, was subjected to ODS (aq MeOH) column chromatography, followed by reverse phase HPLC (aq CH_3CN) to afford **1** and **2** as active principles (yields: **1**, 6.7 x 10^{-3}; **2**, 8.2 x 10^{-3} % wet weight).

The more active compound, (*Z*)-narain (**1**)[3] showed a prominent ion peak at *m/z* 231 corresponding to a formula of $C_8H_7O_6S$ in the negative HRFAB mass spectrum (Δ +1.2 mmu). However, the ^1H and ^{13}C NMR spectra (DMSO-d_6) exhibited signals for 17 protons and 11 carbons, which suggested that **1** was a salt. The presence of *N, N*-dimethylguanidinium ion as the cation was straightforward from NMR data [two amino groups at δ 7.07 (4 H, br. s), an *N, N*-dimethyl goup at δ 2.93 (6H, s)/δ 37.7, and a guanidium carbon at δ 156.7 (C1')]. This was consistent with HMBC correlations[3] and spectral data of an authentic sample (Tokyo Kasei Organic Chemicals, Tokyo). The anion was a (*Z*)-ethenyl-β-sulfate [δ 5.15 (d, *J*=8.1 Hz, H-α)/δ 106.7 (Cα); δ 6.50 (d, *J*=8.1 Hz, H-β)/δ 137.3 (Cβ)] substituted by a 3, 4-dihydroxyphenyl [δ



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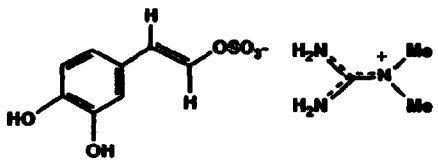

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7.04 (s, H-2)/δ 115.8 (C2); δ 6.62 (d, *J*=8.3 Hz, H-5)/δ 115.1(C5); δ 6.74 (d, *J*=8.3 Hz, H-6)/δ 119.9 (C6); δ 8.76 (s, 3-OH); δ 8.73 (s, 4-OH)] group. Connectivity of these units was secured by an HMBC experiment.[3] The structure of the anion was also supported by the UV [207.0 (ε 16800), 259.5 (8800), and 299.0 nm (3500)] and IR [1200 cm^{-1}] spectra. In order to confirm the total structure, the anion and cation were separated by passing **1** through a CM-Toyopearl column; 3, 4-dihydroxystyryl sulfate was obtained from the water eluate, while *N, N*-dimethylguanidine from the aq NH_3 eluate. Consequently, the structure of **1** is as shown. The less active compound, (*E*)-narain (**2**)[4] had spectral data almost superimposable on those of **1**, except for ^1H NMR signals appropriate for the *E*-geometry of the double bond.

(*Z*)-Narain (**1**) as well as the anion alone induced larval metamorphosis in *H. roretzi* at a concentration of 5 μM, while no larvae in the control group underwent metamorphosis. This suggested that **1** was ten times more active than urochordamine A.[1b] (*E*)-Narain (**2**) and its anion were active only at higher concentrations (50 μM). However, *N, N*-dimethylguanidine sulfate was inactive at a concentration of 50 μM. Therefore, the anion moiety plays the major role as the metamorphosis inducer and the stereochemistry of the double bond is important for activity.[5]

N, N-Dimethylguanidinium salts of sesterterpene sulfates are known from marine sponges, *Coscinoderma*[6a] and *Ircinia* spp.[6b] Interestingly, the anions have been found to inhibit hatching of sea urchin embryos. They were isolated from a marine sponge of the genus *Jaspis* collected off the coast of Kochi Prefecture.[7]

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References and Notes

1. a) S. Tsukamoto, H. Hirota, H. Kato, and N. Fusetani, *Tetrahedron Lett.*, **1993**, *34*, 4819-4822. b) S. Tsukamoto, H. Hirota, H. Kato, and N. Fusetani, *Experientia*, in press. c) S. Tsukamoto, H. Hirota, H. Kato, and N. Fusetani, *Fish. Sci.*, in press.
2. Narain was coined from "narai", which denotes the northeast wind among local fishermen. A very strong "narai" was blowing during collection of the sponge.
3. **1**: IR ν_{max} (KBr) 3320, 3210, 1650, 1250, and 1200 cm^{-1}. UV λ_{max} (MeOH) 207.0 (ε 16800), 259.5 (8800), and 299.0 nm (3500). ^1H NMR (DMSO-d_6) δ 2.93 (6 H, s, 2 x NMe_2), 5.15 (1 H, d, *J*=8.1 Hz, H-α), 6.50 (1 H, d, *J*=8.1 Hz, H-β), 6.62 (1 H, d, *J*=8.3 Hz, H-5), 6.74 (1 H, d, *J*=8.3 Hz, H-6), 7.04 (1 H, s, H-2), 7.07 (4 H, br. s, 2 x NH_2), 8.73 (1 H, s, 4-OH), and 8.76 (1 H, s, 3-OH). ^{13}C NMR (DMSO-d_6) δ 37.7 (q, 2 x NMe_2), 106.7 (d, Cα), 115.1 (d, C5), 115.8 (d, C2), 119.9 (d, C6), 126.9 (s, C1), 137.3 (d, Cβ), 143.8 (s, C4), 144.6 (s, C3), and 156.7 (s, C1'). HMBC crosspeaks: H-2/C4, C6, and Cα; H-5/C1 and C3; H-6/C2, C4, and Cα; H-α/C2, C6, and Cβ; H-β/C1 and Cα; 3-OH/C2, C3, and C4; 4-OH/C3 and C5; NMe_2/C1'. HRFABMS (negative, PEGsul matrix) *m/z* 230.9976 (calcd for $C_8H_7O_6S$, Δ +1.2 mmu).
4. **2**: IR ν_{max} (KBr) 3340, 3230, 1650, 1240, and 1110 nm^{-1}. UV λ_{max} (MeOH) 212.5 (ε 14200), 261.5 (7800), and 305.0 nm (3000). ^1H NMR (DMSO-d_6) δ 2.93 (6 H, s, 2 x NMe_2), 5.80 (1 H, d, *J*=13 Hz, H-α), 6.50 (1 H, d, *J*=8.0 Hz, H-6), 6.62 (1 H, d, *J*=8.0 Hz, H-5), 6.66 (1 H, s, H-2), 6.96 (1 H, d, *J*=13 Hz, H-β), 7.08 (4 H, br. s, 2 x NH_2), 8.73 (1 H, s, 4-OH), and 8.80 (1 H, s, 3-OH). ^{13}C NMR (DMSO-d_6) δ 37.7 (q, 2 x NMe_2), 110.8 (d, Cα), 112.1 (d, C2), 115.8 (d, C5), 116.8 (d, C6), 126.7 (s, C1), 139.0 (d, Cβ), 144.0 (s, C4), 145.3 (s, C3), and 156.7 (s, C1'). HMBC crosspeaks: H-2/C3, C4, C6, and Cα; H-5/C1, C3, and C4; H-6/C2, C3, C4, and Cα; H-α/C2, C6, and Cβ; H-β/C1 and Cα; 3-OH/C2, C3, and C4; 4-OH/C3, C4, and C5; NMe_2/C1'. HRFABMS (negative, PEGsul matrix) *m/z* 230.9955 (calcd for $C_8H_7O_6S$, Δ -0.8 mmu).
5. Fertilization of the ascidian *H. roretzi* was also inhibited by **1** and **2**; **2** was more active than **1**. They were weakly antibacterial against *Flavobacterium marinotipycum* ATCC 19260 and *Alteromonas nautica* IAM 12920.
6. a) L. V. Manes and P. Crews, M. R. Kernan, D. J. Faulkner, F. R. Fronczek, and R. D. Grandour, *J. Org. Chem.*, **1988**, *53*, 570-575; b) A. E. Wright, P. J. McCarthy, G. K. Schulte, *J. Org. Chem.*, **1989**, *54*, 3472-3474.
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